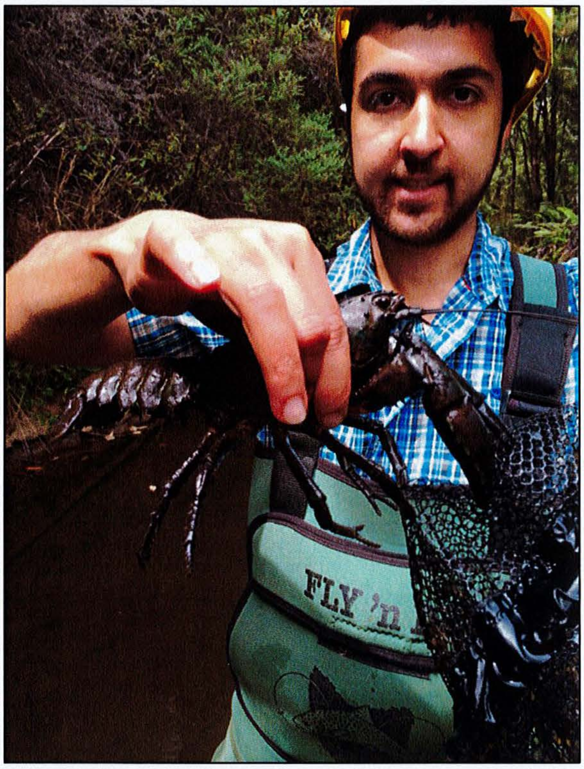
Honours thesis:
Improving study designs for assessing forestry impacts on the giant freshwater crayfish, *Astacopsis gouldi.*



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Declaration

I hereby declare that the presented material in this thesis is my own original work. Any contribution of published literature to my argument is cross-referenced and their opinions are acknowledged in the form of citations. Therefore, I declare that I have not illegally taken credit for any other student's or researcher's arguments.

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Date

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ii. Acknowledgements

My first and foremost thanks for making this research possible goes to my two primary supervisors, A/Prof Leon Barmuta (UTAS) and Dr. Amy Koch (FPA). I learned so many new skills from Leon and Amy they would not fit on this page if I described them. And yet I am nowhere near as brilliant as these two excellent scientists. I am very thankful for the encouragement and patience you have shown around me especially while things didn't go too well in the field; at first. Thanks to your good advice, I was finally able to pull it off! It was an honour to work with you. I am also thankful to my co-supervisor Prof Peter Davies for providing a lot of information about crayfish and how to prepare for field work. Thanks also goes to my field trainers Dr. Laurence Cook and Dr. Alastair Richardson!

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iii. Abstract

This survey aimed at correlating the abundance of Astacopsis gouldi, the world's largest freshwater crayfish, with two different plantation types in Northern Tasmania. After a pilot study revealed inherent difficulties in relying on conventional methods to select suitable sampling sites, selection criteria were refined and ultimately improved through the use of species distribution modelling (MaxEnt). Accumulated mean annual run-off and mean annual rainfall stood out as important in the model and helped to reduce the proportion of intermittent streams in the data set. Analyses conclude that there is no observable plantation effect that correlates with crayfish abundance; however, this should be taken with caution because the sample size was too small to detect a potential effect on crayfish abundance. As a result, approximately 15-18 sites are recommended to be used per tested group. A classification tree further suggests that the presence of undercut banks, log jams and submerged logs might constitute important meso-habitat features that should require further analysis in the future. The study is thus more recommendative in nature and should assist future researchers to develop effective sampling strategies to address the difficulties inherent in assessing crayfish abundance in plantation streams.

Chapter 1 Literature review

Anthropogenic land-use as drivers of freshwater habitat degradation and crayfish decline: a critical review of the contemporary state of scientific knowledge

Key words: Freshwater crayfish, land-use, habitat degradation, sedimentation, riparian vegetation, pollution, alternative methods

Literature review abstract

About one third of the world's crayfish species are listed as threatened and human land-use practices are largely to blame for the observed population declines. Humans modify land to extract or grow resources to sustain the world's growing population and this manifests itself in the form of widespread habitat degradation. With the assistance of some key ecological principles, this review seeks to identify how physical and chemical habitat degradation affects crayfish behaviourally and physiologically. It is revealed that alterations resulting in a loss of in-stream habitat through sedimentation and removal of riparian vegetation produce the greatest negative response. Pollution is another potent factor that impairs the physiological function of key processes in crayfish. However, the reviewed body is relatively sparse, which raises the need for further research in attempt to improve our understanding of how human land-use causes species displacement and population decline.

Introduction

They are regarded one of the world's most important keystone species in freshwater systems (Reynolds & Souty-Grosset 2011), yet surprisingly little is known about these cryptic animals: Crayfish. Where they occur, these decapods maintain the health and function of freshwater ecosystems and thus occupy a crucial trophic position. Although their keystone function is widely recognized in scientific literature, only a relatively small body of literature is devoted towards assessing the impacts of anthropogenic forms of land-use on crayfish habitat and how this in turn affects their physiology and ecology. Key contributers to crayfish decline include anthropogenic habitat degradation involving land-use practices such as agriculture, water management, mining, logging and urbanization (see Fig.1). Even the remaining presented factors (Fig.1) are in some way influenced or exacerbated by human activity. Ignoring the fact that this figure lists the impacts as single factors and that 3 countries hardly constitute a global representation, it seems to indicate that crayfish are relatively well researched and that the key mechanisms drivers of species decline have been clearly identified. While this seems to apply on a general level the exact effects of land-use impacts are unknown on a species-specific level. To illustrate this point further, the following example is presented in figure 2 by Almerao *et al.(* 2015)/ It is almost impossible to fail to notice the omni-present abbreviation "DD" standing for "data-deficient" and indicates that assigning an accurate conservation status is impossible as a result of limited ecological knowledge. This problem is not just common to South America, as will be discussed further on. Much more research has been targeted at crayfish as bio-indicators to test water quality (Reynolds & Souty-Grosset 2011), instead of actually determining how these are impacted physiologically. As a result, there is a clear need to identify where the knowledge gaps in scientific literature lie and where attention should be redirected to in future studies in order to gain more insight into the primary factors contributing towards crayfish decline around the world. Before this can be done, however, several key concepts must be introduced and reviewed.

Species	List	Current conservation status (category/criteria)	Conservation status (category/criteria) suggested
Parastacus brasiliensis (von Martens, 1869)	LEFAE/RS* (Marques *et al.*, 2002) IUCN Red List (Buckup, 2010c)	Vulnerable (VU) Near Threatened (NT)	**Data Deficient (DD)**
Parastacus defossus Faxon, 1898	IUCN Red List (Buckup, 2010d)	Data Deficient (DD)	**Near Threatened (NT)**
Parastacus laevigatus Buckup and Rossi, 1980	IUCN Red List (Buckup, 2010e)	Data Deficient (DD)	**Data Deficient (DD)**
Parastacus pilimanus (von Martens, 1869)	IUCN Red List (Buckup, 2010f)	Least Concern (LC)	**Data Deficient (DD)**
Parastacus varicosus Faxon, 1898	IUCN Red List (Buckup, 2010h)	Data Deficient (DD)	**Data Deficient (DD)**
Parastacus saffordi Faxon, 1898	IUCN Red List (Buckup, 2010g)	Data Deficient (DD)	**Data Deficient (DD)**
Parastacus nicoleti (Philippi, 1882)	CCDNAC/CH**(Bahamonde *et al.*, 1998) Rudolph and Crandall (2007) IUCN Red List (Buckup, 2010a)	Vulnerable (VU) Vulnerable (VU - B1b(i, iii)) Data Deficient (DD)	**Data Deficient (DD)**
Parastacus pugnax (Poeppig, 1835)	CDNAC/CH**(Bahamonde *et al.*, 1998) Rudolph and Crandall (2007) IUCN Red List (Buckup, 2010b)	Vulnerable (VU) Vulnerable (VU - A3cd) Data Deficient (DD)	**Data Deficient (DD)**
Virilastacus araucanius (Faxon, 1914)	CCDNAC-CH**(Bahamonde *et al.*, 1998) Rudolph and Crandall (2007) IUCN Red List (Buckup, 2010j)	Data Deficient (DD) Vulnerable (VU - B1ab(iii)) Data Deficient (DD)	**Vulnerable (VU - B1ab(iii))**
Virilastacus retamali Rudolph and Crandall, 2007	Rudolph and Crandall (2007) IUCN Red List (Buckup, 2010k)	Endangered (EN - B1ab(iii)) Data Deficient (DD) Endangered (EN - B1ab(iii))	**Endangered (EN - B1ab(iii))**
Virilastacus rucapihuelensis Rudolph and Crandall, 2005	Rudolph and Crandall (2005) IUCN Red List (Buckup, 2010l)	Endangered (EN - B1ab(iii)) Data Deficient (DD)	**Critically Endangered (CR - B1ab(iii))**
Virilastacus jarai Rudolph and Crandall, 2012	Rudolph and Crandall (2012)	Critically Endangered (CR - B1ab(iii))	**Critically Endangered (CR - B1ab(iii))**
Samastacus spinifrons (Philippi, 1882)	CCDNAC-CH**(Bahamonde *et al.*, 1998) Rudolph and Crandall (2007) IUCN Red List (Buckup, 2010i)	Data Deficient (DD) Vulnerable (VU - A3cd) Data Deficient (DD)	**Data Deficient (DD)**

Figure 2: Conservation status of South American parastacids (Almerao et al. 2015)

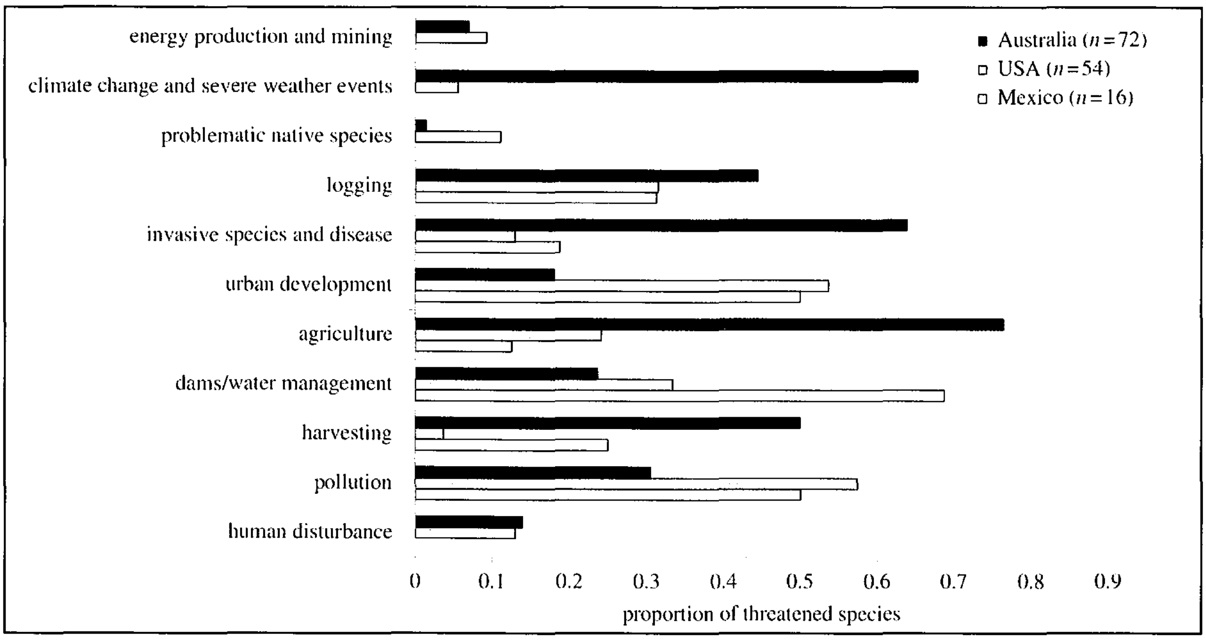

***Figure 1**. Main causes of crayfish decline in 3 selected world regions (Richman et al. 2015)*

Framework of discussion

It is impossible to know how anthropogenic habitat degradation affects basic ecological and physiological characteristics without exploring some fundamental aspects of crayfish ecology. The understanding of this topic is important because physiological stress can be accompanied by reduced fecundity, recruitment or increased mortality as will be discussed further on. A loss of key ecosystem services to other organisms is the logical consequence of crayfish decline, furthering the importance of such research. This literature review will then attempt to outline which natural factors are associated with idealized habitat quality. A basic breakdown of this aspect is summarized on the left-hand side of the presented flow-chart (Fig. 3). Several of the illustrated characteristics must fall together in order to provide refuge and sufficient food for crayfish, which ultimately help to maintain crayfish populations.

On the other side stand the habitat degrading factors. The bold-printed text represents some key land-use practices which have the force to modify crayfish habitat. Urbanization is also an important stressor of freshwater systems, however much less research exists on this topic and hence will not be incorporated into the immediate discussion. Physical habitat modification can be divided into two forms based on how they act in the environment.

Red arrows indicate factors where the physical transformation of freshwater systems through bank-side logging and water regulation leads to increases in sedimentation, temperature and primary productivity. Purple arrows are representative of point and non-point source pollution which alters water chemistry (acidification), heavy metal concentrations and nutrient loading. The latter is a fundamental driver of eutrophy (Camargo & Alonso 2006, Pärn *et al.* 2012). It is also important to remember that the various land-use impacts do not occur in isolation from other external pressures (dashed arrows). Interactions may change the degree of interference with crayfish, therefore interactions will only play a secondary role in the ecological part of this review. The objective of the presented flow-chart is to provide the context in which the various stressors emanating from land-use practices affect crayfish ecology and physiology. Meta-analysis tables are presented to summarize what recent research has found concerning physiological stress which may be associated with population decline. In its totality, therefore, this review aims to uncover the mechanisms underlying an observable species decline as a result of land-use practices and to test the certainty of the claims made by scientists through addressing biases and knowledge gaps in the literature.

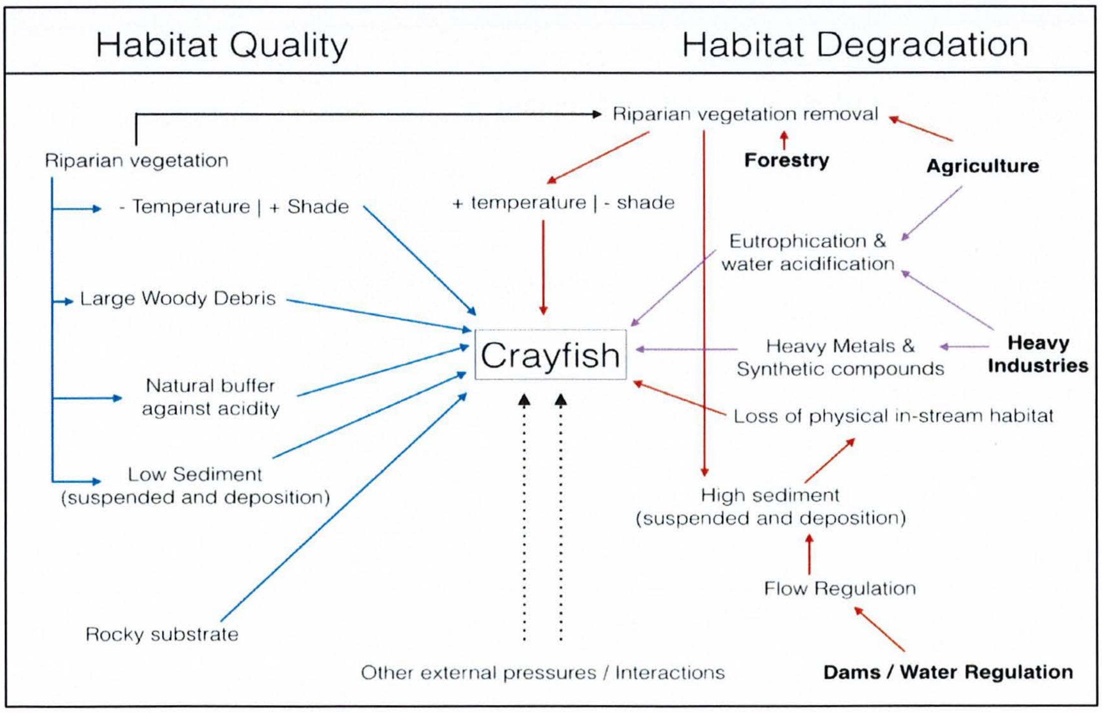

Figure 3. *Flow chart detailing the framework of the review. Blue arrows affect crayfish and habitat positively, while red and purple arrows are associated with degrading factors. Bold printed terms represent some types of land-use. Both positive and negative impacts act on crayfish in conjunction with other external pressure.*

Crayfish Ecology

Distribution & diversity

From the magnificently coloured painted devil crayfish (*Cambarus ludovicianus*) to the heavily armoured Murray River crayfish (*Euastacus armatus*): Freshwater crayfish around the world come in all kinds of shapes, colours and sizes. To date, 650 species have been discovered and about 5-10 new species are described every year (Reynolds & Souty-Grosset 2011). According to Crandall & Buhay (2008) the vast majority of freshwater crayfish are narrowly distributed in geographic space as a result of very specific habitat and climatic needs. As a result, crayfish are thought to be susceptible to habitat degradation.

Freshwater crayfish can be divided into three families: Astacidae, Cambaridae and Parastacidae (Crandall & Buhay 2008). With just 39 species world-wide, Astacidae are the least diverse and occur in Europe, north-western reaches of the North American continent and also eastern Asia (Richman *et al.* 2015). The Cambaridae are the most diverse family, containing > 50% of the world's freshwater crayfish species {Crandall & Buhay 2008, Guiaşu 2009}. Cambarids are native to the south-eastern United States (Fig.4). However, one species of this family, *Procambarus clarkii*, is extremely adaptable and invasive, and is now found in many freshwater streams around the world as a result

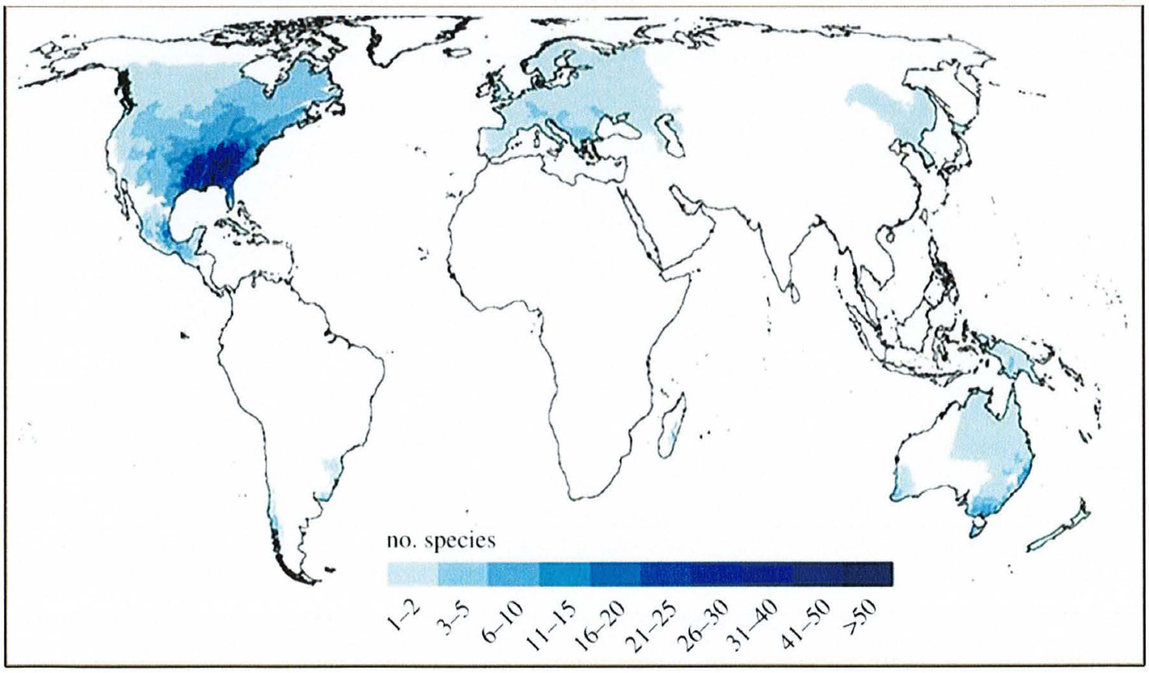

Figure 4: Global crayfish diversity. Darker areas are indicative of higher species diversity {Richman, 2015 #37}.

of deliberate and accidental introductions (Reynolds & Souty-Grosset 2011). Lastly, the Parastacidae are exclusively restricted to the southern hemisphere. Most parastacid species are climatically adapted to temperate regions in Australia, New Zealand and southern South America (Fig.4). However, tropical parastacids can be found in north-eastern Australia, Papua New Guinea and the highlands of Madagascar (Fig.4). The world's largest freshwater invertebrate, *Astacopsis gouldi*, endemic to Tasmania, is also part of the Parastacidae. Continental Africa is devoid of native crayfish species; however, introduced species such as *Cherax destructor* occur there as well (Moore *et al.* 2013). On a global scale, about one-third of the world's crayfish are listed as endangered although as mentioned previously taxonomic and other ecological information is limited Moore *et al.* (2013) exemplify this trend by stating that while an average of 3.4 new cambarids were described between 1972 and 2007, detailed ecological information was only assigned to 0.63 species per year. This, again, highlights the need for a larger body of species specific research.

Crayfish ecology and core habitat requirements

Freshwater crayfish play key ecological roles in aquatic systems. For example, burrowing crayfish are considered as important ecological engineers because their tunnel excavations help to re-oxygenate adjacent soil layers and improve its quality for plants. Empty burrows are taken over by other animals as temporary dwellings, such as frogs (Heemeyer *et al.* 2012). The vast majority of the world's extant crayfish species are detritivorous or omnivorous introductions (Reynolds & Souty-Grosset 2011).These contribute towards a higher concentration of fine particulate organic matter (FPOM) in the water column through sloppy feeding and bioturbation. FPOM constitutes an important food source for other macro-invertebrates and helps to promote biodiversity. Studies also suggest that crayfish exert trophic control on algae introductions (Reynolds & Souty-Grosset 2011). Another feature associated with feeding is that crayfish keep waterways clean through consuming decaying material. Lastly, they themselves constitute an important food source for fish and even for humans.

Freshwater crayfish are relatively specific when it comes to habitat choice (see blue arrows in flow chart for graphic representation). Generally, these animals tend to occupy shaded waters, where the canopies of trees limit water temperature and primary productivity. Furthermore, riparian vegetation plays four other fundamental roles. Firstly, detritus from the riparian zone constitutes an important part of crayfish diet (Giling *et al.* 2009). Secondly, the physical presence of large woody debris in stream water also is a habitat creating force as it is closely associated with the formation of pools and ground cover. This creates habitat complexity and provides important refugia against predation especially for juveniles (Adams 2014). Additionally, large woody debris slows down water velocity and helps to protect in-stream habitat from extreme flooding events (Allan 2004). Rocky substrate affects the hydrology in a similar way to large woody debris and also serves as an important refugium for crayfish (Johnston & Robson 2009). The third key role which riparian vegetation plays is its ability to reduce sedimentation and prevent chemical compounds from reaching waterways (Dosskey *et al.* 2010, Pärn *et al.* 2012). Direct plant uptake and processes such as denitrification in the riparian zone restrict the input of pollutants and macro-nutrients (Ledesma *et al.* 2013, Pärn *et al.* 2012, Zhang *et al.* 2010). Pollutants have the ability to affect crayfish physiologically (refer to tables 2 and 3), therefore the removal of toxic pollutants in riparian zone is an important process. Lastly, shade cast by tree canopies limits light availability and restricts eutrophication (Burrell *et al.* 2014). As a crustacean, it is also vital for crayfish to have access to sufficient concentrations of calcium from which they can grow new exoskeletons (Edwards *et al.* 2014).

In summary it can be stated that crayfish habitat is characterized by clean water, where riparian zones are extensive and sufficient in-stream habitat exists. Crayfish tend to relatively resistant towards short-term environmental change and even impaired water quality (Demers *et al.* 2006). This characteristic allows them to be used as suitable bio-indicators of pollution. Crayfish are advantaged over other aquatic animals in that they have the ability to breathe oxygen from air and can leave degraded catchments if forced to Reynolds & Souty-Grosset (2011). However, when environmental degradation is widespread and long-lasting these animals must endure those conditions. What exactly happens to crayfish in such situations is going to be explored in the subsequent sections.

Physical and chemical habitat degradation and its impacts on crayfish

As introduced previously, physical habitat degradation refers to alterations to the riparian zone and factors affecting the hydrology of rivers, particularly through sedimentation. In contrast, chemical habitat degradation refers to the input of organic and inorganic pollutants from point and non-point sources. When reconsidering the aforementioned core habitat requirements of crayfish, it becomes apparent that many of these impacts create sub-optimal habitat for crayfish. Agents and consequences of

Environmental factor	Effects	References
Sedimentation	Increases turbidity, scouring and abrasion; impairs substrate suitability for periphyton and biofilm production; decreases primary production and food quality causing bottom-up effects through food webs; in-filling of interstitial habitat harms crevice-occupying invertebrates and gravel-spawning fishes; coats gills and respiratory surfaces; reduces stream depth heterogeneity, leading to decrease in pool species	Burkhead & Jelks 2001, Hancock 2002, Henley et al. 2000, Quinn 2000, Sutherland et al. 2002, Walser & Bart 1999, Wood & Armitage 1997
Nutrient enrichment	Increases autotrophic biomass and production, resulting in changes to assemblage composition, including proliferation of filamentous algae, particularly if light also increases; accelerates litter breakdown rates and may cause decrease in dissolved oxygen and shift from sensitive species to more tolerant, often non-native species	Carpenter et al. 1998, Delong & Brusven 1998, Lenat & Crawford 1994, Mainstone & Parr 2002, Niyogi et al. 2003
Contaminant pollution	Increases heavy metals, synthetics, and toxic organics in suspension associated with sediments and in tissues; increases deformities; increases mortality rates and impacts to abundance, drift, and emergence in invertebrates; depresses growth, reproduction, condition, and survival among fishes; disrupts endocrine system; physical avoidance	Clements et al. 2000, Cooper 1993, Kolpin et al. 2002, Liess & Schulz 1999, Rolland 2000, Schulz & Liess 1999, Woodward et al. 1997
Hydrologic alteration	Alters runoff-evapotranspiration balance, causing increases in flood magnitude and frequency, and often lowers base flow; contributes to altered channel dynamics, including increased erosion from channel and surroundings and less-frequent overbank flooding; runoff more efficiently transports nutrients, sediments, and contaminants, thus further degrading in-stream habitat. Strong effects from impervious surfaces and stormwater conveyance in urban catchments and from drainage systems and soil compaction in agricultural catchments	Allan et al. 1997, Paul & Meyer 2001, Poff & Allan 1995, Walsh et al. 2001, Wang et al. 2001
Riparian clearing/canopy opening	Reduces shading, causing increases in stream temperatures, light penetration, and plant growth; decreases bank stability, inputs of litter and wood, and retention of nutrients and contaminants; reduces sediment trapping and increases bank and channel erosion; alters quantity and character of dissolved organic carbon reaching streams; lowers retention of benthic organic matter owing to loss of direct input and retention structures; alters trophic structure	Bourque & Pomeroy 2001, Findlay et al. 2001, Gregory et al. 1991, Gurnell et al. 1995, Lowrance et al. 1984, Martin et al. 1999, Osborne & Kovacic 1993, Stauffer et al. 2000
Loss of large woody debris	Reduces substrate for feeding, attachment, and cover; causes loss of sediment and organic material storage; reduces energy dissipation; alters flow hydraulics and therefore distribution of habitats; reduces bank stability; influences invertebrate and fish diversity and community function	Ehrman & Lamberti 1992, Gurnell et al. 1995, Johnson et al. 2003, Maridet et al. 1995, Stauffer et al. 2000

way to rice fields. Another example from Australia showed that crayfish burrow density was lowest in areas where riparian vegetation was cleared and cattle grazing was extensive March & Robson (2006). This indicates that *Geocherax gracilis* actively avoids areas with a high degree of habitat modification. This notion is supported by Loughman *et al.* (2012) who found a very similar pattern with burrowing *Cambarus thomai* and *Fallicambarus fodiens* in American floodplains. The aforementioned examples illustrate that altering or logging riparian zones to make way for agriculture creates sub-optimal habitat for crayfish. A closer look at figure 6 helps to identify the key differences between riparian zones dominated by grasses or woody plants. One important factor distinguishing the two types is the fact that canopy cover influences water temperature, particularly during summer. Recent research suggests that crayfish are sensitive to increased temperatures (table 1).

Lyons, Trimble, and Paine

TABLE 1. Relative Benefits of Grassy Versus Woody Riparian Vegetation for Small Streams in Grassland/Savannah Areas of Central North America.

Management Aspect	Grassy Vegetation	Woody Vegetation
Bank Stability, Channel Morphology, and Erosion	Less bank erosion; greater trapping of suspended sediment; narrower channels, more undercut banks and pools	Better stabilization of severely eroding banks; wider channels, more diverse substrates
Cover for Fish	More undercut banks, overhanging vegetation, aquatic macrophytes	More large woody debris
Terrestrial Runoff and Subsurface Inputs	Better assimilation of phosphorus	Better assimilation of nitrogen; uptake of nutrients from deeper subsurface waters
Hydrology	Less local flooding; higher baseflows	Reduced downstream flooding
Water Temperature	Unknown; more studies needed	Less variable and lower summer temperatures
Organic Matter and Primary Production	Greater primary production; more algae, macrophytes	Greater organic matter inputs; less less chance of excessive primary production
Macroinvertebrates	Higher per-unit-area abundance and biomass, more herbivores	Greater overall abundance (?), more shredders and detritivores
Fish	Better habitat in some cases, with higher trout abundance; fewer beaver; easier fishing in spring and fall	Better habitat if high summer temperatures or excessive primary production are problems

Figure 6*: Comparison of riparian vegetation from paper by (Lyons et al. 2000).*

These studies demonstrate that an increase in water temperature results in sluggishness and reduced feeding activity in crayfish: a sign of stress. Reduced feeding may compromise the normal function of important biological processes such as ecdysis, reproduction or immuno-competence. For instance Jiravanichpaisal (2006) showed that infectivity to white-spot syndrome virus increased at higher temperatures. Increased temperatures are also related to acidity and facilitate eutrophication; however, this aspect will be discussed in the subsequent section on chemical pollution.

Another factor in which the removal of riparian vegetation can affect crayfish negatively stems from is the loss of woody debris (Figure 5). As mentioned earlier, crayfish not only depend on allochthonous detritus as a food resource but also on large woody debris as a refuge against predation. Wooded stream reaches may vary considerably in terms of dead wood they receive from stream-side vegetation. For instance, Woldendorp & Keenan (2005) state that the proportion of above-ground biomass of large woody debris was 18% in native forests, while a meagre 4% found its way to the forest floor in eucalypt plantations. Leaf litter nutritional quality is also of great importance for macro-invertebrates, therefore the replacement of native trees with exotics can be quite significant (Boyero *et al.* 2012). These examples further suggest that the vegetation communities of the riparian zone fundamentally dictate the amount of nutritious leaf litter and woody debris can reach streams and improve crayfish habitat quality. When large woody debris becomes limiting crayfish exhibit an interesting response (Table 1). Two recent studies showed that when detritus became a limiting food resource, crayfish responded by shifting their diet towards algae. This resulted in a more rapid growth rate, particularly in juveniles, but at the same time it shortened their longevity. Parkyn & Collier (2004) also found that crayfish biomass in pasture streams exceeded that found in forested ones although the former showed a slightly lower density. The researchers attribute the large biomass and faster growth rate to the higher nutritional content of algae compared to that of leaf litter. However, Giling *et al.* (2005) caution from over-interpreting their results due to the fact that crayfish from only one stream were sampled and that more field feeding trials were needed to support the existing results.

Impact	Crayfish Species	Response	Authors & Year	Type of study	Location
Impoundment / Sedimentation	*Procambarus sp., Cambarus sp., Orconectes sp.*	Lower relative abundance, sedimentation restricts burrowing	Adams (2013) Dyer *et al* (2015)	Field survey (CPUE), Lab	USA
Vegetation clearance	*Cherax destructor, Paranephrops planifrons*	higher growth rate, shift to autochthonous food sources	Giling et al. (2009) Parkyn *et al.* (2002)	Lab, 2 year field study	Australia, New Zealand
Increased Temperature	*Euastacus sulcatus, Pacifastacus leniusculus Astacus astacus*	Sluggishness, reduced feeding Reduced immunity against disease	Bone *et al. (*2014) Jiravanichpaisal *et al.* (2004) Johnson *et al. (*2014)	Radio-telemetry (150 days field survey), Lab	Australia, UK Sweden
Loss of habitat complexity	*Cherax destructor Pacifastacus leniusculus*	Increased aggression, particularly among juveniles	Baird *et al.* (2006) Olsson & Nystrom (2009)	Lab, 1 month field test	Australia Sweden
Low water level / Water abstraction	*Astacus astacus*	Higher juvenile mortality during peak activity time	Tulonen *et al.* (2010)	Manipulated outdoor experiment	Finland

Table 1*: Meta-analysis of habitat degradation and its effects on crayfish behavior and physiology*

A further physical factor associated with the systematic removal of riparian vegetation is sedimentation (Fig. 5). In-filling of habitat through sediment deposition as result of bankside erosion is a critical factor affecting a wide variety of aquatic organisms (Allan 2004). The effects of sedimentation can further be exacerbated through other human land-use practices such as water regulation (Friedl & Wuest (2002), Rolls *et al.* 2012). Areas downstream from dams and stream reaches affected by water abstraction typically suffer from modified-flows and altered sediment deposition regimes, which reduced the abundance of some cambarid species (Adams 2013), table 1. The overall loss of habitat complexity as a result of sediment in-filling is met with some profound behavioural responses in crayfish. For example the lacking in-stream refugia increased intra-specific aggression in *Cherax destructor* as individuals were forced to compete for shelter (Baird et al. 2006). This can be particularly devastating for juveniles in particular and affect recruitment. Olsson & Nystrom (2009) ound the same effect in European *Pacifastacus leniusculus* in a field trial. In some instances limited in-stream habitat causes crayfish to seek other forms of cover. Research by Adams (2014) on *Procambarus* and *Orconectes* species showed that these animals did not hesitate to use objects, such as trash, as a shelter. Further research on water sedimentation shows that burrowing activity is also reduced (Dyer *et al.* 2015), suggesting that heavily sedimented areas do not represent suitable habitat for burrowing crayfish species. Another study on water regulation by Tulonen *et al.* (2010) showed that when water levels were artificially lowered juvenile *Astacus astacus* mortality increased because this made them more susceptible to predation. These examples all suggest that sedimentation is a very particular threat that can affect crayfish on many levels and facilitate species decline.

Therefore, as it currently stands, land-use practices which alter the structure and species composition of the riparian zone, but also affect the hydrology of streams, seem to have the greatest impact freshwater crayfish.

Impact of chemical habitat degradation

Chemical habitat degradation occurs primarily in the form of water pollution and changes to water chemistry. According to Schwarzenbach *et al.* (2006), whether a compound can be defined as a pollutant depends on its input, distribution and fate (longevity) in a given system, and the overall effects it exerts on organisms. Different land uses have different pollution signatures. For instance acid mine drainage, effluents and wastewater containing heavy metals are discharged directly into rivers by mines (Allert *et al.* 2009). Because the area of input is confined to a small location, this type of pollution is referred to as point-source pollution. In contrast, non-source pollution is a temporally and spatially continuous form of pollution involving inputs of macro-nutrients, fine sediments and other chemical compounds. This type of pollution is common in agricultural and silvicultural contexts. How these main forms of pollutants affect water quality and crayfish will be described in the following section.

Point source pollution

Surprisingly little information has been gathered on the effect of heavy metals on freshwater crayfish in natural settings. Crayfish are presumed to be resistant to moderate levels of heavy metal contamination and much attention has been attributed to using these animals as bioindicators (e.g. Reynolds & Souty-Grosset 2011). The existing studies, of which the vast majority are lab-based, have provided valuable insight into how heavy metals and other toxic compounds are absorbed into crayfish bodies and affect them physiologically. Table 2 shows some of the most common researched contaminants in freshwater systems. Some elements such as aluminium, chromium and copper seem to not affect crayfish strongly. Copper, in particular, is thought to be tolerated at medium-high concentrations because it is used as a key oxygen binding agent in hemocyanin - the crayfishs' respiratory pigment. This comes in spite of the fact that Lahman *et al.* (2015) found that elevated copper concentrations impaired the ability of *Procambarus clarkii* to detect food; an effect which could have other behavioural implications. Another factor which protects crayfish from moderate levels of pollution is the ability of the hepatopancreas to effectively detoxify organs and tissues and mitigate further physiological complications. Elements such as fluoride accumulates most readily in the exoskeleton and periodic ecdysis can prevent an excessive fluoride build-up in other tissues. However, during inter-moult, when shedding is impossible and fluoride accumulation occurs rapidly this can be accompanied by other behavioural constraints. Research by Aguirre-Sierra *et al.* (2013) found that the most significant change was that *Austropotamobius pallipes* exhibited a reduced escape response, followed by reduced feeding activity and mortality with increasing fluoride concentrations in the various treatments. While a reduction in tail-flip response might not necessarily kill the animal, it may make it susceptible to predation by being unable to escape. Further research is needed to know how severe significant fluoride accumulation is for crayfish populations.

Pollutant **Heavy metals & Fluoride**	**Species**	**Findings**	**Author**	**Method**	**Location**
Aluminium	*Pacifastacus leniusculus*	localized toxicity / inflammation of hepatopancreas	Woodburn *et al.* (2011)	Lab test	UK
Cadmium	*Procambarus clarkii*	Decrease in tail-flip response at high concentration Increase in claw-raise behaviour	Wigginton *et al.* (2010)	Lab test	USA
Chromium	*Procambarus clarkii*	Uptake in blood, but uncertain effect	Anderson *et al.* (1997)	Field test	USA
Copper	*Procambarus clarki* *Orconectes rusticus*	Slow accumulation rate Impaired chemical orientation	Anderson *et al.* (1997) Lahman *et al.* (2015)	Field test, Lab test	USA
Fluoride	*Austropotamobius pallipes*	High concentration = reduced escape response & feeding increased mortality	Aguirre-Sierra *et al.* (2013)	Lab test	Spain
Lead	*Procambarus clarkii*	Accumulation in hepatopancreas increase in ph digestive juices	Anderson *et al.* (1997)	Field test	USA

Heavy metal Interactions					
Selenium - Lead	*Procambarus clarkii*	high exposure (time & concentration) caused paralysis	White *et al.* (2012)	Lab test	USA
Lead - Zinc - Nickel	*Orconectes hylas*	increased mortality in proximity to point-source	Allert *et al.* (2009)	Field test	USA

***Table** 2: Meta-analysis of chemical degradation and its effects on crayfish behavior and physiology. Studies including interactions can be found in the bottom half of the table. First part on page 18, second part above.*

In contrast, heavier elements seem to have a much stronger impact. *Moderate* concentrations of cadmium and lead showed quite marked physiological and behavioural changes (see table 2). While some studies decided to measure the effect of a single trace elements, others tested how metal interactions might affect crayfish (Table 2, bottom). These studies report high mortalities and paralysis, which highlights the need for additional studies analyzing these interactions in greater detail.

Non-point source pollution

The Nitrogen:Phophorus ratio is a fundamental driver of eutrophication in freshwater ecosystems Smith (2003). Generally, these elements are readily used by primary producers and hence limit the availability of this macro-nutrient in the water column (Adams 2013, Dyer *et al.* 2015). However, the use of fertilizers containing nitrogen and/or phosphorus and nitrogen fixing crops often results in excess nutrient run-off (Pärn *et al.*, 2012).

Stream-side vegetation may prevent the spill-over of macro-nutrients into freshwater systems either through direct uptake or by providing suitable conditions for denitrifying bacteria. By contrast, and as touched on earlier, clearing riparian vegetation facilitates bank-side erosion leading not only to an increase of suspended sediments and base cation concentrations (Ledesma, *et al.* 2013), but also to an increased ability of nitrogen to reach water through ground-water or overland flow Pärn et al. (2012).

Impacts of hypoxia and water acidification on crayfish

According to research, presented earlier, crayfish have the ability to switch their diet to algae when exposed to a spike in algal biomass (Giling *et al.* 2009 ; Parkyn *et al.* 2002) and may exert trophic control on it (Reynolds & Souty-Grosset 2011). This suggests that crayfish can tolerate eutrophication to a certain extent. Excessive algal mass, however can change the water chemistry to the detriment of freshwater organisms. If eutrophy exceeds a certain biomass, death among primary producers stimulates the growth of oxygen depleting bacteria. Hypoxic conditions arise when oxygen becomes limiting in the water column and this could pose a threat to crayfish. This idea was tested in two studies which both concluded that *Austropotamobius pallipes* were tolerant of hypoxic conditions despite a disruption in the ion exchange process (Table 3). The field study conducted by Lyons & Kelly-Quinn (2003) picked up a lower relative abundance in the sampled areas and attributed this phenomenon to the decreased water quality associated with hypoxia. Excess nitrogenous compounds such as nitrite can further affect respiration rate and induce physiological stress in crayfish (Meade et al. 1995). The other effect of nitrogenous compounds (nitrate, nitrite, ammonia) is that they can acidify water through donating hydrogen ions into the system (Camargo & Alonso 2006).

This phenomenon is particularly visible in European and North American rivers, where acid rain and centuries of agricultural land-use have caused stream pH to decrease between 4.5 and 6 (Camargo & Alonso 2006). Crayfish, possessing calcareous exoskeletons should face increasing difficulties to survive in streams with elevated acidity levels. This notion is supported by recent studies (table 3). Crayfish can tolerate much lower pH levels than some fish species (pH 6), despite being disadvantaged in such conditions Olsson *et al.* (2006). This may be beneficial, according to these researchers, because crayfish can escape predation and eliminate this additional stressor. However a lab study by France (1993) found that at pH 5.1 a high number of crayfish eggs did not harden sufficiently enough to prevent direct embryonic mortality. In addition almost half of the juveniles perished at this low pH level. This example shows that crayfish recuitment becomes markedly impaired when faced with water acidification.

Table 3 (below): *Meta-analysis of the effects of nitrogen pollution and acidification on crayfish physiology and ecology.*

Other chemical forms of water pollution	Species	Findings	Authors	Methods	Location
Eutrophication/ Hypoxia	*Austropotamobius pallipes*	Tolerant despite disruption in ion exchange process, but reduced relative abundance	Demers *et al.* (2006) Lyons & Kelly-Quinn (2003)	Long-term monitoring (5y) Lab test	Ireland, Central Europe
Nitrite	*Cherax quadricarinatus*	Reduced oxygen consumption rate	Meade *et al.* (1995)	Lab test	USA
pH = 5.1	*Orconectes virilis*	36% reduction in egg hardening increased embryo mortality 45% mortality of juveniles	France (1993)	Field test and lab test	Canada
pH < 6	*Paranephrops planifrons*	tolerant, reduced predation by trout	Olsson *et al.* (2006)	Field test	New Zealand

Limitations & knowledge gaps

Thus far, a review of the effects of land-use and crayfish habitat and on the species themselves has revealed some important behavioural and ecological responses. For instance research showed that crayfish actively avoid areas where in-stream habitat is lacking or where the clearance of riparian vegetation induced complex physical and chemical changes in stream water (table 1). Lab studies provided some context to show that pollutants initiate physiological stress and how this may affect crayfish recruitment and other key biological functions (table 2 & 3). In its entirety, the recent literature is relatively sparse and in essence, too few studies have taken research questions further. This section will now address some key limitations and the knowledge gaps in this scientific discipline that must be covered in order to provide a more detailed overview of the effects of land-use on crayfish.

Sparsity of data and insufficiency of study types

The limited number of studies is problematic for several important reasons. Tables 1-3 all show that in most cases one or two studies focused on a particular issue and their methods varied quite considerably between once-off lab studies and 5-year field surveys. Lyons & Kelly-Quinn (2003) stated, however, that only one day each year, although it was the same day, was used to assess the catch per unit effort and determine the relative abundance of crayfish in hypoxic conditions. Year to year variability in abiotic and biotic factors may not have been accounted for sufficiently, therefore the true effect of hypoxia on crayfish abundance may have eluded the researchers. Multiple measurements throughout the year could have fixed the problem which highlights the necessity for seasonal surveys rather than strictly yearly ones. From the presented tables (1-3) it also becomes apparent that roughly half of the studies did not take place in a natural setting. And between these studies, very different approaches were taken to estimate the impact on crayfish. This indicates that replication is a major problem in this scientific discipline, as essentially no study picks repeats the same methods in a different area for comparison. Most of the presented field studies operated in one catchment which suggests that variability between streams is unknown. Furthermore, scale is another important factor to consider because an environmental stressor may vary depending on whether it acts at a regional, catchment, reach or habitat scale Burskey *et al.* (2010). This and other uncertainties revolving variable abiotic factors could explain why several measured characterists turned up with trends rather than with statistically significant results (e.g. Tulonen *et al.* 2010). Therefore, without a more widespread and accurate replication of these field studies it is difficult to validate the existing claims. Essentially what is needed to combat this paucity in knowledge are additional, more powerful study types. These could incorporate before and after field studies or long-term monitoring programs at sensible temporal and spatial scales.

Another apparent limitation is that too much attention is currently devoted to test the suitability of crayfish as bioindicators of water quality rather than measuring the direct outcomes of pollution accumulation in crayfish. In addition these bioindicator-studies rely on lethal crayfish take and dissection to determine pollution concentration in tissues (Hothem *et* *al.* 2007, Mason *et al.* 2000, Nakayama *et al.* 2010), therefore the effect of trace elements on live animals is not measured. Only one study seemed to investigate the impacts on recruitment France (1993) by assessing water quality impacts on egg viability and juvenile mortality. A combination of lab and field studies of this type is needed to understand how the various life-cycle stages respond to a particular environmental stressor. Furthermore, studies should also address the impact of urbanization on freshwater crayfish through inputs of synthetic compounds, pesticides and endocrine disruptors (Chen *et al.* 2006, Fong *et al.* 2014, Houde *et al.* 2011). This work is in its infancy and is likely to become more important in the future.

Bias in species selection

Using tables 1-3 there seems to be a study bias towards commonly researched species. These include *P. planiphrons, A. astacus, P. leniusculus, A. pallipes, C. destructor, Orconectes sp.* and the 'world-invader' *P. clarkii*. The bias towards these species may additionally stem from the fact that they are relatively common in the streams they occur in and are generally well-studied compared to their much rarer counterparts. As introduced earlier, many crayfish species around the world lack sufficing ecological data (Almerao *et al.* 2015), particularly more so in non-industrialized countries. Jones *et al.* (2007)'s study was the first large-scale survey to assess land-use impacts on Madagascar's endemic crayfish species. This also highlights the needs for additional research on tropical species. However, using a few, more frequently studied species introduces a new problem: As mentioned earlier, *Procambarus clarkii*, is an extremely adaptable species which has invaded streams around the world successfully. As a result it may exhibit a higher resistance against stressors than other crayfish species. Using this species as a proxy to infer impacts on all extant crayfish has to, therefore, be taken with great caution. False inference of the effects of habitat degradation on crayfish could severely inluence the effectiveness of management practices.

It is therefore necessary to shift the baseline away from a few common species to a wider selection of freshwater crayfish in natural versus impacted settings. To do this, however, additional biological and ecological knowledge about these species must be acquired before impact responses can be determined experimentally.

Interactions and multiple factors

About half of the reviewed papers were conducted in a lab setting (tables 1-3). Though some valuable insights of physiological stress were gained, these studies are limited because they typically test one or two factors. However nature is much more variable and many external pressures from land-use and other sources act on crayfish at any given time Richman, et al. 2015. It is generally unknown what the relative strength of each existing stressor is and which of those more likely induces population decline. For instance, while only two of the mentioned studies discussed interactions between trace elements and found significantly stronger crayfish responses, a much larger body of literature focused on measuring lethal doses of one element and gradually increase its concentration to detect a behavioural response. In a natural setting, crayfish are never exposed to just one element, which raises the need for further studies assessing more complex interactions. Even in a natural setting, the relative contribution of each potential stressor acting on the environment has the potential to obscure the observed trends.

For instance trout predation may be a stronger force than water pollution as implied by Olsson *et al.* (2006). Other stressors such as disease may be increased in the presence of others as was demonstrated by Jiravanichpaisal *et al.* (2004),(table 1). Therefore, within the the context of land-use impacts on freshwater systems, much more work needs to incorporate the various existing interactions between into experimental design in order to obtain a more accurate representation of how crayfish behave in their combined presence. Modelling population responses in the presence of these interactions can be a viable tool to describe trends associated with crayfish decline.

Conclusion and future directions

This review revealed two key aspects about the state of contemporary research concerning the impacts of anthropogenic land-use on freshwater crayfish. Firstly, current research suggests that the loss of physical in-stream habitat and water pollution are significant pressures affecting relative abundance and recruitment. Secondly, although the claims seem robust, the various limitations affecting these studies raises doubt about whether the findings are truly representative of all extant crayfish. Before such general claims can be made further research is needed. This includes taxonomic knowledge, particularly through identifying life-histories and determining how anthropogenic stressors affect the entire life-cycle. Future studies should concentrate on replication and long-term field surveys so that a comparative platform is created. The effects of urbanization on freshwater crayfish, a factor which is currently under-studied, also requires considerable attention, particularly because its direct effects remain highly speculative. In this study temperature was also shown to be a predictor of physiological stress and may prove to become more relevant in the future especially with respect to recent climate change. Constructing models to predict crayfish response to increasing temperature and other potential stressors may help to define precise, tailored management plans. However, to reach this far considerably more research is needed in order to determine how human impacts can be mitigated and allow crayfish populations to prosper far into the future.

Chapter 2 - Introducing the research question

As identified in the literature review, crayfish respond differently to various environmental stressors and have been shown to be relatively robust to water contamination and various other forms of land-uses (e.g Demers et al. 2006). However, long-term studies and repetitive surveys were identified to be lacking, making it difficult to generalise the impact of land-use on these animals. This is further made difficult because various forms of land-use each might affect abundance differently. One way to gain insight into how well crayfish fare in modified landscapes is to conduct an abundance survey, coupled with species distribution modelling. Such studies can assist in selecting suitable sample sites in which a better estimate of abundance can made. This methodology was employed as a first on the Tasmanian giant freshwater crayfish, Astacopsis gouldi, in order to assess abundance in contrasting silvicultural catchments.

2.1 - Study species and deeper research context

The giant freshwater crayfish, *Astacopsis gouldi (A. gouldi),* or tayatea, is the world's largest freshwater invertebrate, with historical records suggesting that individuals could attain a mass of up to 6 kg and live for approximately 40 years (Horwitz 1994). The species is endemic to northern Tasmania and is thought to occur mostly in low elevations (up to 400m). There, these animals inhabit cool, shaded streams with a wide availability of food and shelter. However, their home range also coincides with areas used by humans for industrial purposes. Habitat modification and illegal poaching in these areas of contact are estimated to have led to considerable declines of this species by more than 50% (Walsh & Doran 2010). As a result, the species was listed as a threatened species and is now under government protection; but habitat modification is still widespread in its native range (Walsh & Doran 2010).

Additional research is required in order to ultimately benefit the recovery of crayfish in modified landscapes. Several older studies have centered around describing the life history of *A. gouldi*, while newer reports have focussed more on crayfish abundance in forestry streams. However, these reports have not assessed how crayfish abundance correlates with contrasting plantation types.

In early 2015, one attempt was made to answer this question. A survey was designed with the aim to assess crayfish abundance in class 2 and 3 streams in pine and eucalypt plantations. 'Class 2 and 3' is a forestry size classification and refers to streams with a catchment area of 50 ha – 500 ha. 30 sites such sites would be sampled for crayfish. In preparation of the survey, background knowledge and GIS spatial layers was sought to guide the selection of relevant criteria for surveying this species. Apart from catchment size, other variables given a higher importance was geology and factors relating to the crayfish range. For example, a higher abundance of crayfish was linked to 'basaltic', spring-fed streams. In addition, according to the Conservation of Freshwater Ecosystems Values (CFEV) database from which crayfish range information was obtained, it is assumed that every stream section which is above 400 m in elevation and in small headwater streams (Strahler order 1) do not harbour *A. gouldi*. After sites were filtered out based on the aforementioned few criteria, these sites were visited.

However, the survey came to a grinding halt early on: four out of eight sites were found to be completely dry. It was uncertain whether the size classification was a problem or whether predictions of the crayfish range were lacking information. To avoid running out of funds as a result of low sampling success, it was necessary to test other methods that could be used to refine site selection criteria. Species distribution modelling was the method of choice to address this question because such models use presence information to infer relative habitat suitability. The usefulness of this method and how to interpret its predictions is presented in Chapter 3. It also forms the basis on which Chapter 4 sites were selected. However, the main aim of this research, that is to correlate *A. gouldi* abundance with two different plantation types, remains unchanged.

It is worth mentioning at the start that no plantation effect was observed on crayfish abundance. Some emergent difficulties that may have influenced the outcomes and how to address these issues in future studies are presented in Chapter 4. Therefore, the value of this research lies in providing recommendations and insights into emerged difficulties for future studies seeking to answer questions relating to crayfish abundance in plantations.

Chapter 3– Refining the distributional models for *Astacopsis gouldi*

3.1 - Introduction

In the past decade, species distribution modelling has become an increasingly popular tool to model the past, present and future distribution of species and has been used to address important questions relating to topics such as species invasiveness (e.g. Feria & Faulkes 2011) or range shifts in response climate change (e.g. Dyer *et al.* 2013). However, it is not the aim of this study to research similar effects. Rather species distribution modelling constitutes a potential way to refine current site selection criteria which, if successful, can assist in reducing time, costs and other resources which is involved in searching for this species.

Broadly speaking there are two types of species distribution models. One type involves possessing information on both presences and absences of the target species at many locations. These types of models are typically stronger because one can measure and know why a species is absent from a certain area. In the other, more common type of SDM, information on absences is lacking; therefore, presence-only models must be used to identify potentially suitable habitat (Philipps *et al. 2006,* Elith *et al.* 2011). The algorithms of these presence-only modelling tools work by populating the background with pseudo-absence points and compare these with presence points. However, the models can be influenced through bias in the location records, and predictions of habitat suitability must be treated with caution while absence information is unknown (Elith *et al.* 2011).

Currently, there are several species distribution modelling tools available which work with presence-only information: Bioclim, Domain, GARP and MaxEnt. A comparative review by Hernandez et al. (2006) concluded that, while these each have their strengths and weaknesses, the strongest and most consistent performance was demonstrated with MaxEnt, when using presence-only data.

Basically, MaxEnt acts as a Quasi-poisson model, where the number of occurrences are a function of environmental variables (Merow *et al.* 2013). It is used to predict relative habitat suitability across a study area or landscape which is divided into grid cells (Philipps *et al.*, Merow *et al.* 2013). Based on the input data and user-specific constraints, the algorithm estimates the most uniform probability distribution which is also referred to as a 'distribution with maximum entropy' (Philipps *et al.* 2006). It does so by comparing a set of specified random background points from the landscape to the species locations and, based on the model it builds, assigns a probability of occurrence value to each grid cell in the defined landscape (Philipps *et al.* 2006). The final result of MaxEnt's calculations is a map in which high and low suitability areas are identified. It also provides summaries of model performance, species preference curves for input variables and the contributions of each variable to the model which help to interpret the results biologically.

MaxEnt also benefits its users in various other ways. For instance, MaxEnt was found to be the most consistent in its predictions even at low sample sizes (Hernandez *et al. 2006*). In addition, categorical variables require no transformation, while variable interactions pose little difficulty for the algorithm to deal with (Phillips *et al.* 2006). However various researchers claim that MaxEnt is vulnerable to bias and would lose predictive power if its interpretations are extrapolated to unsampled regions (Peterson Townsend *et al.* 2007, Phillips *et al.* 2006). However, the same authors state that MaxEnt performs better than GARP across densely sampled landscapes and this has been confirmed in other studies (Elith *et al.* 2011, Philipps *et al.* 2006).

The north of Tasmania has been sampled relatively well for *A. gouldi* with more than a thousand data points in the Natural Values Atlas (NVA), therefore it was expected that MaxEnt could reconstruct a more realistic picture of this species' distribution.

However, spatial environmental predictor variables are often themselves modelled values and may not entirely reflect the 'natural' status quo. Such instances and the influence of bias may reduce MaxEnt in its predictive ability. However, bias can be confronted either by filtering in the data set or by constructing a bias grid for MaxEnt to single out areas in the landscape that may be prone to it (Elith *et al.* 2011). If bias is addressed appropriately, the predictive outcome will be more accurate.

3.1.1 - MaxEnt use in crayfish ecology

In recent years, the number of studies using MaxEnt to model crayfish distribution has grown (see Table 1). Most of these were conducted in the United States and Europe. The only southern hemisphere example was built for an invasive crayfish in Madagascar (Feria et al.), and they focussed on the invasiveness of introduced crayfish (e.g. *P. clarkii*, *P. fallax* , *P. leniusculus*) and predicting areas where these species could establish. Currently, there seem to be no published SDM studies for crayfish in Australia. In general, the species distribution or habitat use literature on Tasmanian crayfish, such as *A. gouldi* is scarce. One attempt to characterize habitat suitability for *A. gouldi* by Hamilton *et al.* (2015) using Bayesian network modelling concluded that geomorphic condition of river beds was more important than elevation; however, more research was required to confirm the ideas presented in this study.

Authors	Year	Location	Species
Dyer *et al.*	2013	United States	*Orconectes leptogonopodus* *Orconectes menae* *Orconectes saxatilis* *Procambarus tenuis*
Endries, M.	2011	United States	Various *Cambarus* and *Orconectes* species
Feria & Faulkes	2011	Madagascar, Europe, North America	*Procambarus fallax*
Gallardo & Aldridge	2013	United Kingdom and Ireland	*Procambarus fallax* *Procambarus clarkii*
Ghia *et al.*	2013	Italy	*Austropotamobius pallipes*
Larson *et al.*	2010	United States	*Pacifastacus leniusculus*
Larson *et al.*	2012	United states	*Pacifastacus leniusculus* *Procambarus clarkii* *Cherax quadricarinatus*
Morehouse *et al.*	2013	United States	Cambarus ludovicianus
Morehouse & Tobler	2013	United States	Orconectes rusticus

Table 2*. Recent literature where Maxent was used to estimate crayfish distribution*

3.1.2 - Aims

There were two aims for this part of my project. The first was to build a more precise predictive model for the distribution of *Astacopsis gouldi* and assess how it differed from the current predictions made by CFEV. The second aim was to assess the influence of observer bias on the model's predictive outcomes. Ultimately, both aims should serve as a guide to refine site selection for subsequent research for this species.

3.2 - Methods

3.2.1 - Location data; preparation and filtration

Over 1000 records of *Astacopsis gouldi* from across its native range were obtained from the Natural Values Atlas (NVA 2015) which is a database including museum records and more recent field observations to which researchers have contributed. There were no duplicate records in the dataset. Some (ca. 20) older records lacked precise geolocations and were far away (> 500 m) from the closest stream section. These points were thus removed from the data set. It is also preferable to use newer records so that these agree with the existing environmental conditions (Phillips 2006). The vast majority of points in the NVA were collected within the past 20 years, therefore they are likely to be representative.

Because MaxEnt is sensitive to bias in the data, it was considered necessary to reduce the potential effects of it. Two bias risks were singled out which have the potential to skew the predictive outcome of the model (Philipps 2006): sampling intensity and observer bias.

High sampling intensity may lead to clustering of observation points in the landscape causing certain ecological factors to stand out more than others, effectively swamping variation within and between groups. This may result in a potential misinterpretation of habitat suitability. To reduce this possibility, clusters of records within 300 m of each other were identified in ArcGIS and reduced to one randomly selected point from each cluster.

Observer bias has various facets, ranging from accessibility of sites to frequently visiting streams for a specific sampling purpose. This problem was addressed by emailing the various contributors to the NVA database and requesting information about the circumstances under which the points where collected. Some of the respondents noted that stream class (a forestry classification based on catchment area), accessibility and the degree of human impact had played a role in site selection and hence dictated where *A. gouldi* were sought. The locations provided by Todd Walsh were used initially because he explicitly avoided biasing his sampling based on any of these criteria. However, Mr. Walsh was biased towards 'natural' sites which meant that disturbed sites and larger, downstream streams were likely under-represented. This yielded more than 800 records (including clusters) of *A. gouldi* in the NVA. Once these records were filtered for clustering just over 130 points were left over. MaxEnt can work well with very low samples sizes (n≥10); therefore, this sample size was considered sufficient.

3.2.2 – Environmental layers

Shapefiles, henceforth referred to as layers, of stream characteristics were obtained from the Conservation of Freshwater Ecosystem Values (DPIW) database. This database consists of modelled data which is used to quantify the condition of all freshwater bodies in Tasmania, including streams. Stream lines in CFEV are derived from a fine-scale digital elevation model, and the stream lines in the study area were imported into ArcGIS and clipped to the existing *A. gouldi* range boundary identified in CFEV. This boundary is based on altitude and the cut-off point is 400 m, where low densities or no crayfish have been presumed. CFEV stream lines contain various environmental data such as stream order, elevation, accumulated catchment area, accumulated mean annual run-off, slope, catchment disturbance and sediment input, to name a few. Additional layers representing vegetation (Tasveg 3.0), geology (Tasmania 1:250.000 resolution provided by the Forestry Practices Authority) and Tasmanian climate variables (listed in Appendix 1; sourced from the Australian Bureau of Meteorology) were imported into ArcGIS as well and also clipped to the *A. gouldi* range boundary. For a full list of all environmental variables used in the model, their source and definitions refer to Appendix Table 1.

3.2.3 - GIS manipulation

Both the un-clustered observation points and environmental layers required further editing in ArcGIS before utilization in MaxEnt. Observation points initially did not fall on the CFEV stream lines; therefore, these were manually moved by a few metres to sit on top of them. This was done so that the algorithm restricts its predictions to stream sections and not the remaining terrestrial landscape. MaxEnt only requires a species name and coordinates (eastings and northings) for the input format. Thus, all other remaining information was removed.

The shapefiles of the environmental variables were in vector format, and this is incompatible with MaxEnt. The program requires environmental layers to be in a gridded raster representation of the data, where each grid cell size and the extent is determined. The extent of each environmental layer must match that of each of the other layers. Each grid cell size was given the size of 25 m x 25 m and the extent, or more precisely the extent of the *A. gouldi* range, was fixed in the ArcGIS 'Environment' settings. Commonly other studies used 1km grid cell sizes or larger. However, Graham & Hijams (2006) demonstrated that model performance was improved with smaller grid cells, therefore the use of comparatively tiny grid cells is justified for use on small streams. In this light, twelve gridded rasters representing environmental variables were created (see Appendix table 1) and imported into MaxEnt's java-based interface together with the observation points.

3.2.4 - MaxEnt modelling criteria

Several conditions were selected to create the Maxent model. The first condition was to use the default setting of 10,000 background points. These are randomly selected pixels from the background landscape representing pseudo-absences which are compared to presence locations in the model (Philipps 2006). Because the presence and background points are chosen at random for testing and training it is possible that the predictions differ from slightly from model to model. In order to validate the model, it is possible to cross-validate multiple repetitions of the model and obtain a consensus model. Therefore, the second condition was to run the model using three-fold cross-validation. When cross-validating, Maxent uses the default amount of subsamples for testing and training in each run. No random seed was selected. In addition model all of the available fitting terms (such as linear, quadratic, hinge) were selected. All other settings were left at the default.

3.2.5 - Measures of model performance and reduction

In MaxEnt, diagnostic plots are created which can be used as a means of gauging model performance. The 'receiving operating characteristic', or ROC curve, provides an overview of how much better or worse a model is compared to random one and how good the model is at predicting presences contained in test samples (Phillips 2006). MaxEnt then calculates an 'area under the curve' value (AUC), where an AUC > 0.5 (on a 0-1 scale) reflects a better than random model. MaxEnt also produces jackknife graphs in which variable contributions to the model can be measured. The variable contributing to the AUC the least and resulting in a no more than a 1% drop in AUC from one model to the next was selectively removed and the model re-run without it. This was done to single out the fewest possible variables with the highest AUC. Once the jackknife suggested substantial drop in AUC (i.e. >> 1%) through the removal of a variable, no more reductions were carried out.

The gridded output map, based on this final model, was imported into ArcGIS, where the predicted suitability values were summarized into ten groups based on their suitability rating (on a percentage scale). This grouping allows clearer optical differentiation between low and high suitability sites. Lastly, Bias was assessed by repeating the aforementioned procedure with un-clustered version on non-Todd Walsh NVA points and a combination of both Todd and non_Todd un-clustered records.

3.3 – Results

3.3.1 - Model reduction

The full model (Model 1, Table 2) includes all of the twelve input variables representing environmental information, and has an AUC value of 0.858 which indicates that the model is much better at predicting presences than a random model would. The removal of slope, solar radiation and catchment disturbance resulted in almost no change in AUC, suggesting that these variables contribute little to the model. Removing vegetation increased the AUC to 0.868 which suggests that it has a slight negative effect on the model. This also occurs when mean annual temperature is removed from the model. However, these increases are quite small, and likely unimportant for the purposes of the first aim. Removing accumulated catchment area from the model caused the AUC to drop slightly. This variable is strongly correlated with accumulated natural mean annual runoff and, if it were used without the run-off layer, it would be the strongest contributor to the model (AUC of 0.844). The removal of stream order resulted in no change in AUC. However, the removal of sediment input increased slightly AUC, suggesting it too exerts a negative effect on the model. Further removals were tested for, but they all resulted in very large drops in AUC, therefore Model 9 was declared to be the strongest.

3.3.2 - Interpretation of Model 9

The summary ROC curve of the 3-fold crossvalidation with Model 9 (AUC = 0.885) a is shown in Figure 1. This model retains accumulated mean annual run-off, mean annual rainfall in Tasmania, elevation and geology. Comparison of the AUC to the black line in Figure 1 denotes how different the model is from a random model: it clearly outperforms random models.

Model Number	Removed variable from previous model	AUC	Difference from previous model
Model 1	Includes all variables	0.858	n/A
Model 2	slope	0.859	0.001
Model 3	solar_radiation	0.859	0
Model 4	catchdist	0.86	0.001
Model 5	tasveg	0.868	0.008
Model 6	mean_temp	0.873	0.005
Model 7	acccatchmentarea	0.869	-0.004
Model 8	stream_order	0.869	0
Model 9	sed_input	**0.885**	0.016
Model 10	removed elevave	0.874	-0.011
Model 11	remove geology	0.871	-0.014
Model 12	remove geology and elevae	0.859	-0.026
Model 13	remove acnmmar; leave in other 3	0.769	-0.116

Table 3. *Summary of Model reduction. Model 9 performed the best out of the tested models and included: accumulated normal mean annual run-off, mean annual rainfall, geology and elevation.*

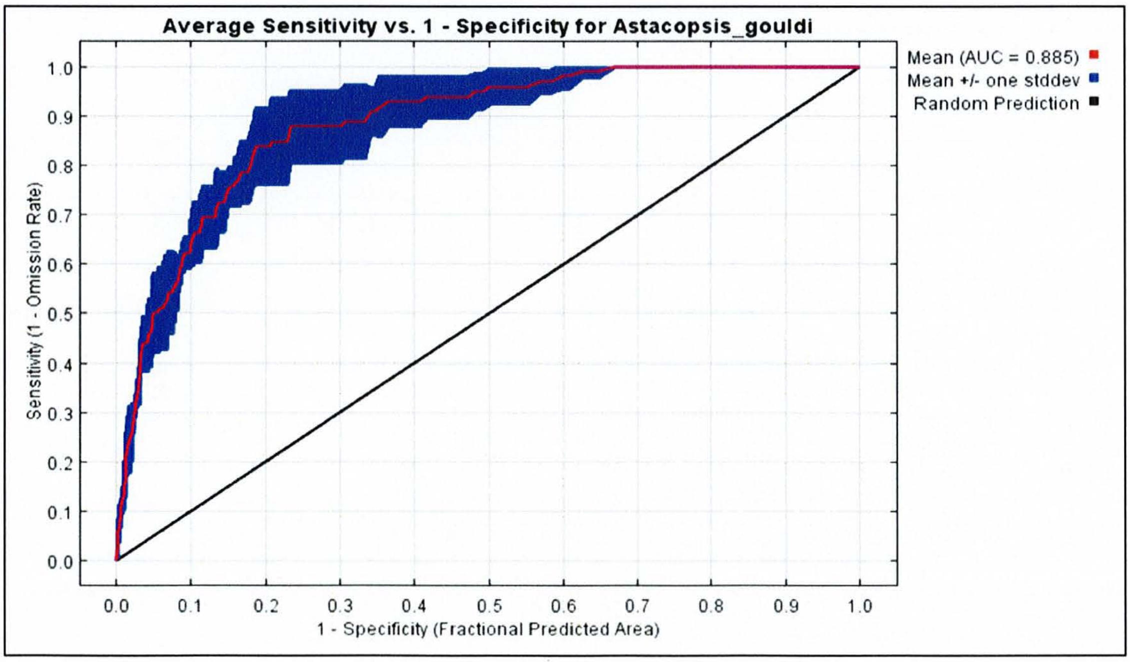

Figure 1. *ROC curve showing that the model is better at predicting presence points (red line) than a random model (black line) over the predicted area. The AUC value of model 9 is 0.885.*

The rank order of the variables in this model was: accumulated normal mean annual run-off (acnmmar), mean annual rainfall, geology and elevation. The jackknife graph (Fig. 2) shows how much a variable contributes towards the AUC. By itself, acnmmar is the strongest contributor (dark blue bar) out of the four variables and its removal from the model would cause significant drop in AUC (teal bar, also evident in Table 2). Conversely, geology (geo_merged_geocodes) holds the least information by itself and would lead to a small drop in AUC if it were removed. The order of contribution according to this is therefore acnmmar, mean annual rainfall, elevation, and geology.

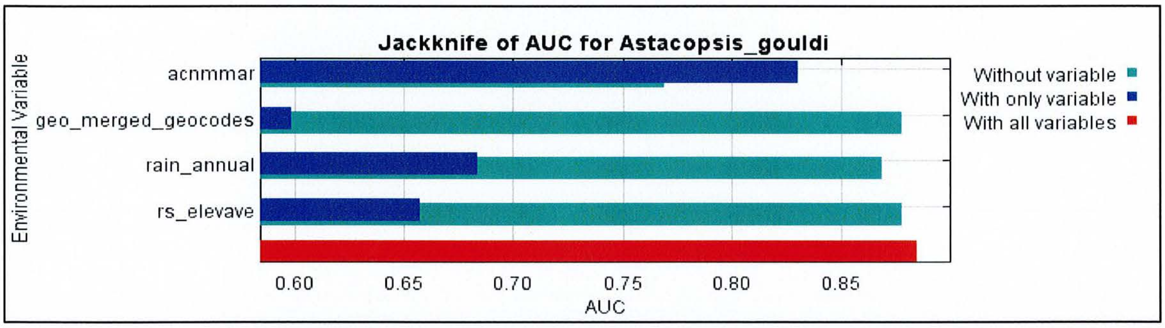

Figure 2. *Jackknife graph showing the relative importance of variables and contribution to AUC (red bar). Blue is a measure of strength of what the AUC would be with just this variable. Green bars show what would happen to the AUC if all* ***other*** *variables are kept in the model.*

Figure 3 shows how probability of presence (red line) varies within the spread of measures contained in each the retained environmental variables. The blue band represents standard errors of predictions. The top left panel shows that *Astacopsis gouldi* are more likely to be detected in areas which receive more than 0.001 ML km^2 $year^{-1}$. It is important to recall that accumulated run-off increases with increasing catchment size. There is a size limit to the size the stream in Northern Tasmania can reach: however, these larger stream reaches appear to have been sampled to a lesser degree. This can be seen in the increased standard errors and higher difficulty to detect *A. gouldi*. The top right panel suggests that groups 1 (schist and siliceous rocks) and 2 (basalt and dolerite) increase the probability of presence. However, based on this scale it can also be suggested that groups 4 and 5 can be moderately associated with presence. Interestingly granite, which was grouped in the 3rd group and is more common is the North-east is given the lowest probability of presence rating. The bottom left panel shows that the probability of presence increases to a maximum of 1000 mm – 1430 mm annual rainfall. Left and right of this optimum probability of presence drops off fast and suggests that *A. gouldi* tend to avoid areas with low and very high annual rainfall. The bottom right panel shows that when elevation increases the probability of presence decreases as well. The optimum lies at about 30m above sea level. This confirms the notion that *A. gouldi* is a low-elevation species. The overall suitability map for this species based on this model is shown in Figure 4.

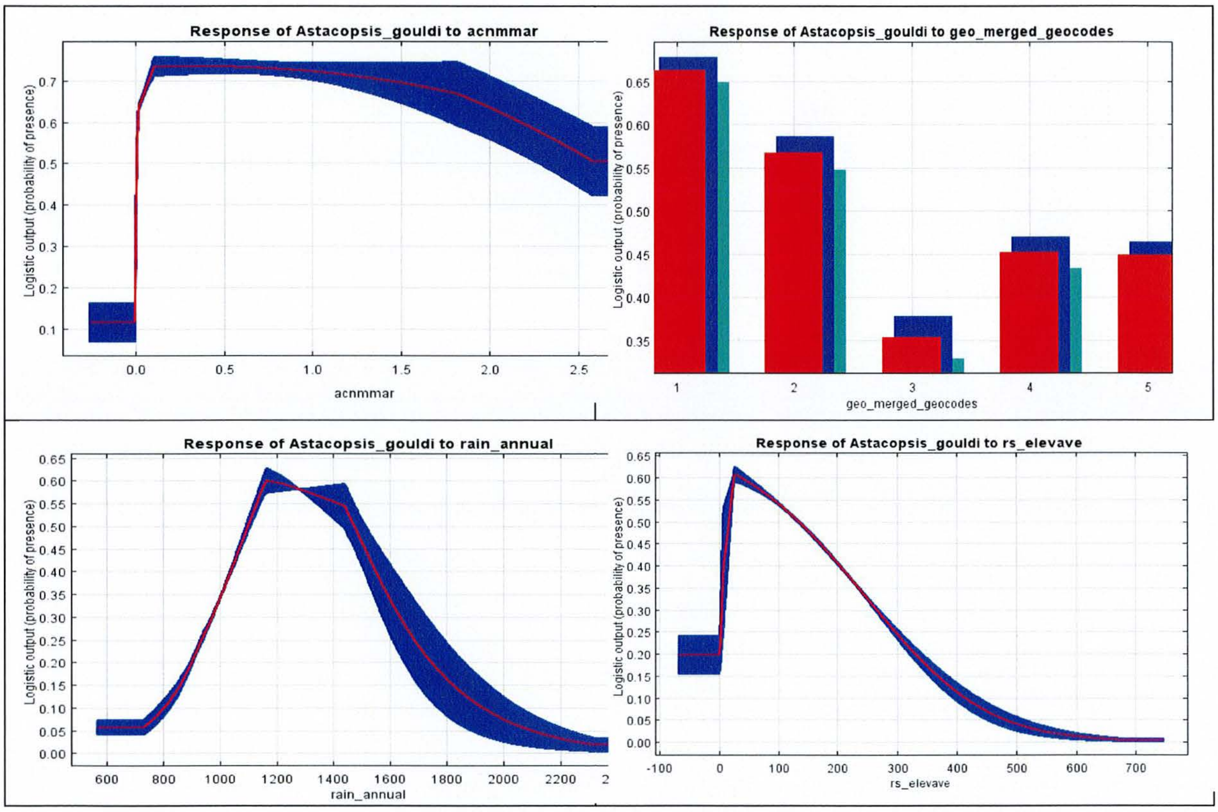

Figure 3. *Red line is the representative of the probability of presence based on input points. Top left: Sharp increase in probability of presence for* A. gouldi *in increasing accumulated normal mean annual run-off (acnmmar). Units are in ML km^2 y^{-1}. Top right. Geology categories (grouped geology types); 1 = schist and siliceous rocks, 2 = basalt/dolerite, 3 = other, 4 = Quartzose sediments, siltstone and mudstone, 5 = loose and fine sediments. Bottom left = Mean annual rainfall in mm y^{-1}, Bottom right = average elevation of river section in meters.*

3.3.3 - Comparison with failed sites

For the purposes of this study, modelling species distribution with MaxEnt appears to be a useful tool in reducing the amount of dry streams which resulted in a much higher number of crayfish detections. The direct comparison between the two surveys shows a stark contrast and is summarized in table 3. Firstly, 50% of streams using the CFEV prescriptions were dry, compared to 4% using the MaxEnt predictions. The only entirely dry stream was encountered east of Sheffield and was given a Maxent suitability rating of 4. It was the highest rating for a stream in that region, therefore, if the model bases its predictions on rainfall and run-off this could mean that the whole area may receive less water than wetter areas such as the Flowerdale catchment. In all other areas almost five times more crayfish were detected relying on the MaxEnt prediction. These results are indicative of a strong improvement over the old model and are thus more suitable for selecting sites.

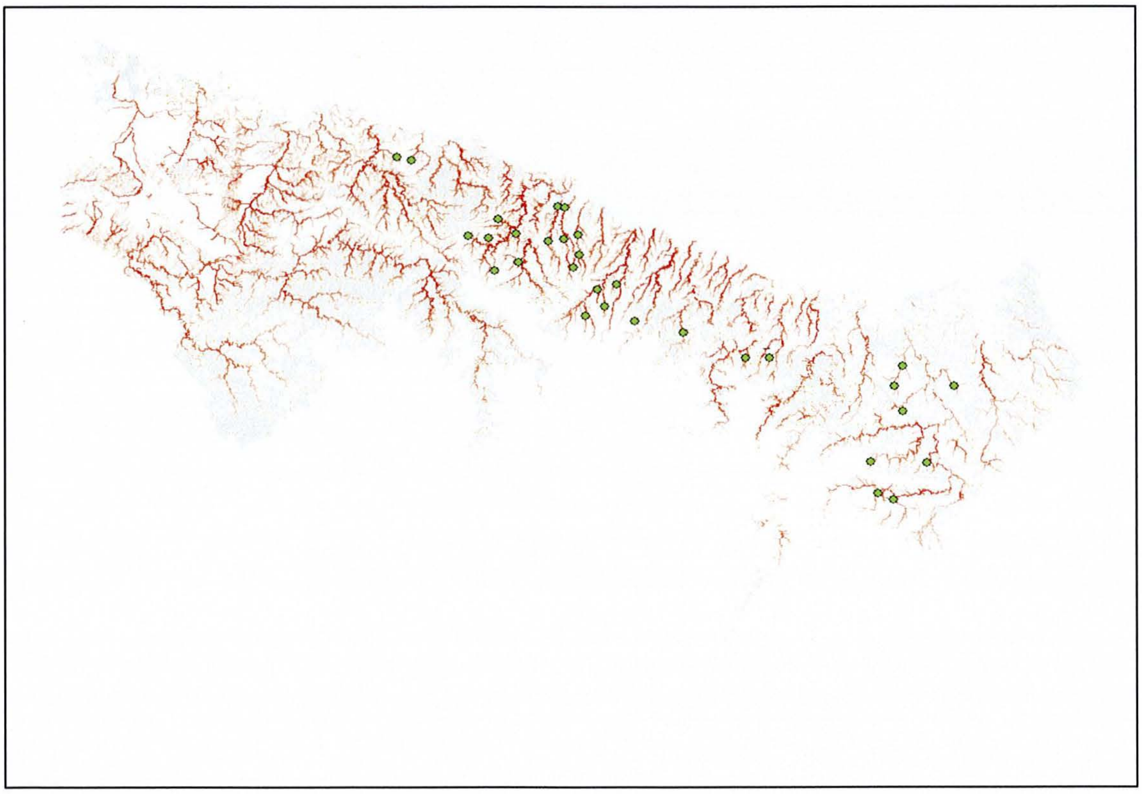

Figure 4. *Suitability map based on final model in the* Astacopsis gouldi *range (Northern Tasmania) in which darker stream 'lines' represent high suitability areas. Conversely, grey areas are estimated to be less suitable for this species. Each point on this map is a pixel which has its own suitability rating. Green points show sample sites plus some additional back up sites that were selected with the MaxEnt method.*

Survey	Number of sites visited	Number of dry sites	Number of crayfish detected
Preliminary field survey	8	4	5
Survey using MaxEnt prediction	23	1	24

Table 3. *Comparison of sites visited versus the number of crayfish detected and the number of dry sites. The MaxEnt method seems to be an improvement over previous estimates, highlighting the usefulness of species distribution modelling for A. gouldi.*

3.3.4 Bias assessment

Model 9 was based on Todd Walsh's data. A similar model was constructed using all the available, un-clustered data points and a third excluding Todd Walsh's observation records and removing clusters. In all three instances acnmmar and mean annual rainfall contribute the most towards AUC. This suggests that these factors are probably be key habitat indicators otherwise their contribution could drop out in any of the other models. However, this is not seen at all. On the lower end of these models, the 3rd and 4th best contributors differ from each other. Stream order and disturbed sites appear more in the non-Todd data and is an artifact of sampling with pre-defined research questions in mind (ie targeting a specific stream order or disturbance level). It results in the lowest AUC score and also the lowest contribution of acnmmar to the score. Vegetation seems to play a minor role in the combined model, while geology seems to have a minor importance. Overall, the model seems to be resistant to bias because the strongest contributors to AUC remain similar across the three predictions.

<table>
<tr><th></th><th colspan="3">Todd records</th><th colspan="3">Combination</th><th colspan="3">Non-todd records</th></tr>
<tr><td></td><td>Full</td><td colspan="2">Reduced</td><td>Full</td><td colspan="2">Reduced</td><td>Full</td><td colspan="2">Reduced</td></tr>
<tr><td>AUC</td><td>0.858</td><td colspan="2">0.885</td><td>0.844</td><td colspan="2">0.834</td><td>0.826</td><td colspan="2">0.824</td></tr>
<tr><td rowspan="4">Variables & Contri-bution</td><td rowspan="4"></td><td>acnmmar</td><td>70.8</td><td rowspan="4"></td><td>acnmmar</td><td>77.8</td><td rowspan="4"></td><td>acnmmar</td><td>66.2</td></tr>
<tr><td>rainfall</td><td>12.4</td><td>rainfall</td><td>13.9</td><td>rainfall</td><td>13.5</td></tr>
<tr><td>geology</td><td>9.6</td><td>tasveg</td><td>4.6</td><td>Stream order</td><td>12.9</td></tr>
<tr><td>elevation</td><td>7.2</td><td>geology</td><td>3.7</td><td>catch_dist</td><td>7.4</td></tr>
</table>

Table 4. *The first major column is a summary of the full and reduced model described earlier. The second column would show these results, if contributor information were not available. The last major column shows what would happen when Todd's observations are removed and a model with the same variables run. The main picture, despite some re-arrangements along the lower end of the top variables, show that accumulated mean annual run-off and rainfall consistently dominate in the models and seem to be useful in predicting suitable localities for sampling.*

3.4 - Discussion

This study revealed that the MaxEnt method was an improvement in terms of detecting *Astacopsis gouldi* when compared to reliance on the CFEV recommendation of suitable habitat. Rainfall and accumulated normal mean annual run-off dominated in the model, suggesting that core habitat is distributed in area receiving enough input of water from various sources to reduce stream intermittency. Filtering out biased points resulted in a higher AUC, but in terms of the top predictions of AUC variable contribution, bias in the NVA data seemed to have a lesser impact. However, previous understanding of the crayfish range may have resulted in some areas being overlooked where presences could be higher than predicted. This may apply at small streams, higher elevations (150m – 450m) or at large, downstream catchments where detectability may decline as stream depth and width increase. Imperfect detection certainly has the power to influence model prediction. However, based on what data is available, this is the best prediction one can obtain by using the existing NVA records and environmental spatial files to construct a habitat suitability model with MaxEnt.

In any case, the usefulness of using this method to improve site selection seems to have been demonstrated. However, the question remains how accurate the input information is and if some of the lesser contributors to the model actually contribute more towards meso-scale processes (e.g. rockiness of stream beds, canopy cover) which the literature confirms to be important for crayfish (Davies et al. 2005a, Davies et al. 2005b, ; Davies *et al.* 2007, Horwitz 1994). In addition, there is no information on species interactions, migration or distinction between habitat use and suitability in juveniles versus adults. The model is thus far from complete, because it does not incorporate this information. Furthermore, this study also did not test whether the low suitability areas, where pseudo-absence is inferred, are indeed sites of low suitability.

In order to gain a better biological understanding of this species distribution model, it is necessary to put the insights obtained into the context of the existing literature. Currently, nine studies have used MaxEnt to estimate habitat suitability. One of these studies by Dyer *et al.* (2013) found that suitability was mostly dictated by winter temperature / precipitation and elevation for three out of four *Orconectes* species, while geology was only found to be important for *O. menae* (sandstone geology). Other studies link habitat suitability to seasonal/annual precipitation (Feria & Faulks 2011, Larson *et al.* 2010), seasonal/annual temperature (Gallardo & Aldridge, Ghia *et al.* 2013, Larson *et al.* 2010, Morehouse & Tobler 2013) and slope (Ghia *et al.* 2013). Only Dyer *et al.* (2013) included geologic and soil descriptors, whereas the others studied species distribution more as a function of climate to address climate change and invasive species related questions. In their discussion, these researchers cite studies in which crayfish distribution was linked to geology (France 1992; Joy & Death 2004; Westhoff *et al.* 2011) but others showed that geology was unlinked (Westhoff, Guyot & DiStefano, 2006).

The results of this study are somewhat ambiguous. While previous research suggests *A. gouldi* presence may be influenced by spring-fed, 'basaltic' streams (Davies *et al.* 2005a,b), the MaxEnt model suggests that schist and siliceous geology was more strongly linked to presence than basalt and dolerite (but not by a large margin). Crayfish also seemed to be moderately detectable even in areas with loose sands and sediment. Rarer geology types (group 3) are uncommon in the Tasmanian landscape and therefore they are also linked to a lower probability of presence. This might suggest that *A. gouldi* is much more a generalist when it comes to the catchment's geologic make-up.

All in all, there are few studies that incorporate a wide range of environmental variables to construct a MaxEnt model for crayfish, therefore comparison to these few and mostly different studies is unhelpful. What remains is to suggest ways with which the current model can be improved.

3.4.1 - Further research

There are several alternatives available to improve the assessment of habitat suitability for *Astacopsis gouldi*. One way includes continuing with MaxEnt because absence data is not available. Another way might involve collecting absence data and use occupancy modelling (Presence software, for example) to avoid using pseudo-absences and get a more concrete picture of crayfish distribution across Tasmania's north. The latter method may assist with extrapolating suitable habitat to the rest of Tasmania, where such information could be used to successfully translocate populations and help to manage this threatened species more effectively. MaxEnt has difficulties extrapolating to unsampled areas despite being able to apply 'clamping' to the data (Townsend Peterson *et al. 2007*). Therefore, in order to extrapolate a model to an unsampled region, it would be wiser to collect absence data.

If continuing with the MaxEnt's presence-only algorithm, one alternative would be to ask the NVA to request collector information of meso-habitat variables. This information could be used in a species-with-data context - an alternative input format in which each point has environmental variables listed in the input table in addition to eastings and northings - and might to fine tune the model. It would also be helpful for collectors to provide information on the age and sex of crayfish. This can be used to model potential differences in habitat use between different age/sex classes. Additionally, incorporating additional layers such as soil structure might help in teasing out relationships between erodibility and crayfish use of undercut banks and the potential to cause sedimentation. Lastly, it is also necessary to assess whether low suitability sites are indeed low suitability sites. This model accuracy test will assist in identifying whether the model over- or underestimates habitat suitability. If this is done properly, it will give researchers more confidence that the model is reliable and is more representative of the species' realized niche.

There are many potential avenues which could lead to a much stronger MaxEnt model. However, the current model is still useful in that it allows better discrimination of habitat suitability than the existing recommendation and benefits the site selection process. It can help to improve the relative success of future research that might be in turn used to improve species distribution modelling for *Astacopsis gouldi*.

Chapter 4 - **Correlations of abundance with contrasting forest practices**

4.1 Introduction

Forestry is identified as one common form of land-use in northern Tasmania and various forest practices have the potential to influence crayfish abundance through sedimentation (e.g Davies *et al.* 2005a, see chapter 1) and streamside vegetation clearance (Giling *et al.* 2009). To reduce the effect of harvesting operations, legislation now requires plantations to maintain stream side reserves, or 'buffer zones', of 30m width (Forest Practices Code 2015). It remains to be seen whether these are adequate in protecting crayfish habitat in plantations.

Within the *A. gouldi* range, two common plantation types include monoculture eucalypts (*Eucalyptus nitens*) and pines (*Pinus radiata*). Hence, the survey was designed to correlate these contrasting plantation types with crayfish abundance through a wide collection of environmental variables.

4.2 - Methods

4.2.1 Site selection

Using the MaxEnt method outlined in Chapter 1 to infer relative habitat suitability, 30 sites with a suitability rating of 30% or higher were selected as sample candidates. Of these, 10 sites were in native forest (control) and 10 sites each in *Pinus radiata* and *Eucalyptus nitens* plantations. All sites were restricted to Tasmania's north-west for sampling efficiency and controls were paired wherever possible with the randomly selected plantation sites. The geographical area studied encompassed the Flowerdale River catchment in the western section until Paramatta Creek and Gog Range streams towards the east.

North-eastern Tasmanian streams were not included because of the legacy of tin mining in the area the markedly different bedrock geology (e.g. granite), which, with the chosen sample size, may confound forest practices effects when compared to north-western streams.

Sites were selected in ArcGIS to have good road access in order to be able to manage to survey two sites a day. The survey itself was carried out over 15 days of field sampling and took place during November (late austral spring). Typically, the first survey took place between 10am and noon, while the second survey typically commenced at about 1pm and lasted until 3pm.

4.2.2 - Field survey

Before the field survey took place, it was determined that juvenile and adult crayfish would be sampled differently. Juvenile crayfish spend most of their time hidden under rocks, boulders and submerged logs (Davies *et al.* 2005a, Davies *et al.* 2005b). Therefore, hand searching was the most effective option to search for juveniles. This involved turning over rocks and logs and hand-netting individuals. Large adults on the other hand may be seen out in the open or can be trapped in ring nets. Where sites had a large number of deep pools (> 1m) up to four baited double ring net traps were deployed along the surveyed stretch. The bait used was blue bait as this was the only bait available for purchase at the local fish and chips shop. Traps were left in the stream for approximately two hours. Though this duration is short, it was necessary in order to get to the survey destinations within the given time-frame.

Detected crayfish were removed from the water by hand net and measures of carapace length (age determination) and sex, as well as coordinates were taken. Sub-adults and adults were defined as individuals having a carapace length of 6 cm or more. Sampling for juvenile crayfish, where possible, took place a short distance upstream (and in an upstream direction) from the trapping area to improve detectability by allowing downstream water movement to clear out sand and silt which might obscure vision. All caught crayfish were released back to where they were found.

A series of other environmental variables were collected in order to measure potential plantation effects on the stream reach which could affect the abundance of crayfish negatively. Two whole sets of measurements were taken at the start and end points of the surveyed transect. For example, one measurement consisted of the proportion of embedded rocks from the total rock count. This was used as measure to quantify sedimentation intensity. A silt score rating based on my personal judgement was also used to quantify sedimentation because the quadrat used was too small to pick up patchy sedimentation in streams. On the other hand, rock cover and organic litter were estimated using the quadrat as it was difficult to estimate the proportion of boulders, cobbles, rocks and pebbles over the survey distance. Submerged logs and logjams were also counted because these might constitute potential refuges for crayfish. Some water measurements were obtained as well, which include pH, temperature, conductivity, water depth and wetted width. Bankside characteristics were also measured and this involved estimating the height of the riparian vegetation, bank slope, proportion of undercut banks and the degree of shading from canopies. Because a densitometer was unavailable, I devised my own way of measuring shading. It involved forming a ring with thumb and index fingers, holding it about 10cm above the eyes and using the other hand to take a picture of the hole and canopy in the field of view with a smartphone. The proportion of area covered by trees was then turned into a percentage cover value. In plantations, the stream-side buffer or reserve distance was measured as well in order to measure plantation proximity to the streams.

In addition, several methods were selected to account for catch per unit effort. Effort was measured as catch per 'survey distance', 'survey time' and catch per 'rock turned over'. However, because it was difficult to keep these measures constant this resulted in poor quantification of effort (all $p > 0.2$), therefore the results are not reported further on.

4.2.3 - Statistical Analyses

The presence or absence of adult and juvenile crayfish abundance were analysed using binomial or quasi-poisson generalised linear models (GLM) respectively. In both analyses, predictor variables for a full model were catchment type (factor with 3 levels: Control, Eucalypt plantation and Pine planation), proportion of site with undercut banks (underc), the number of logjams in a site (logjams) and silt coverage of sites by fine sediments (ordinal factor, 'siltsc', 3 levels: Low = < 1/3 of stream section surveyed with silt cover, Mid= 1/3 - 2/3 silt cover, High = > 2/3 silt cover). These such meso-habitat was considered to be important for crayfish based on literature (e.g. Davies *et al.* 2007).

For abundance data, quasipoisson models were used because of moderate overdispersion in the data. Sample sizes were not large enough to allow the inclusion of interactions or curvilinear terms. Full models were simplified by manual backward elimination using standard analysis of deviance tests. The full model diagnostics suggested a strong influence of site K, but removal of this site did not affect the significancy of the tests and so the analyses here report on the full data set. All analyses were completed in R (R Core Team 2015)

In addition to these analyses, and in light of the low sample size, a power analysis was conducted to detect a difference between forestry practices as large as the largest effect found in the existing data set. The analysis was conducted on the log-transformed scale (to match the log-link in the poisson GLMs) using the 'power.t.test' function in R.

Lastly, a classification tree was constructed to identify which variables were associated with a presence or absence of *A. gouldi* in the surveyed streams as an exploratory analysis to guide future surveys in this region. This was achieved through the use of the 'rpart' package in R (Therneau *et al.* 2015). A large tree was 'grown' to achieve the best prediction of presence with the goal of exploring which of the collected variables at each site might be useful to include into future surveys. Auxilliary outputs from 'rpart' were examined to identify strong contenders for the strongest variable at each node. These are summarised in a table and constitute variables which should likely be prioritized in further research in this region. Therefore, this classification tree was not used to predict any outcome but rather to explore trends that might be worth investigating in the future.

4.3 - Results

4.3.1 - Site details

It was not possible to visit all 30 candidate sites: 7 sites were inaccessible due to road blockages and deterioration and impenetrable walls of blackberry thickets leading up to the streams which were often situated on steep slopes; access to one of the site was prevented because the road led through a commercial quarry. One stream in the Gog Range (labelled Site T) was divided into two sites because the upstream area had been recently replanted and the downstream area recently clear-felled. Upstream sedimentation was much more obvious than the harvested reach, yielding one crayfish observation. Site K (in a pine plantation) was identified as an outlier with 7 larger crayfish found (size range of 5.2 cm – 10.4 cm carapace length). The site had little canopy shading and very high in sediment coverage, but with undercut banks and a few submerged logs. A similar observation was recorded in site R, although with only two adults detected. Other sites were quite variable in terms of rockiness, sediment load and riparian vegetation structure.

In total 23, crayfish were found of which one evaded capture. Table 5 contains some demographic information of the crayfish sampled. Juveniles and young sub-adults (< 6 cm carapace length) were almost entirely observed in riffle streams with small pools, while adults were mostly encountered in larger streams. The only exception of an adult in a riffle was recorded in a control stream in the Gog Range. No adult crayfish were caught in traps and were mostly encountered in the open. 9 out of 23 crayfish found occurred at seven control sites, 13 at 10 pine plantation sites (Figure 5); but about half of those at site K a single crayfish was observed in five eucalypt plantations. This single observation occurred in the only eucalypt stream with an intact riparian buffer with native vegetation. Other riparian buffer zones in eucalypt zones were heavily intruded with blackberry or pine which may be the result of either downstream dispersal or the result of land-use pre-dating the plantation. No crayfish were observed where blackberry bushes proliferated on stream banks. A graphical representation of the crayfish count in the various catchment types is illustrated in Fig.5.

The GLM showed there was no significant difference between contrasting plantation types ($p > 0.5$). The only variable that seemed to significantly correlate with crayfish presence was the proportion of undercut banks in plantations ($p < 0.02$).

	Crayfish number	Mean CPL	Min. CPL	Max. CPL
Juvenile	14	1.92	0.55	4
Male	3	6.55	6.2	9.8
Female	5	7.8	5.2	10.4

Table 5. A. gouldi *demographics of collected sample. CPL = carapace length and adults or older sub-adults are identified as individuals with a CPL of > 6 cm. 24 crayfish were captured in total. One escapee was estimated to have a CPL of 3-4 cm. The sex of this individual is unknown and therefore it is not represented in this table.*

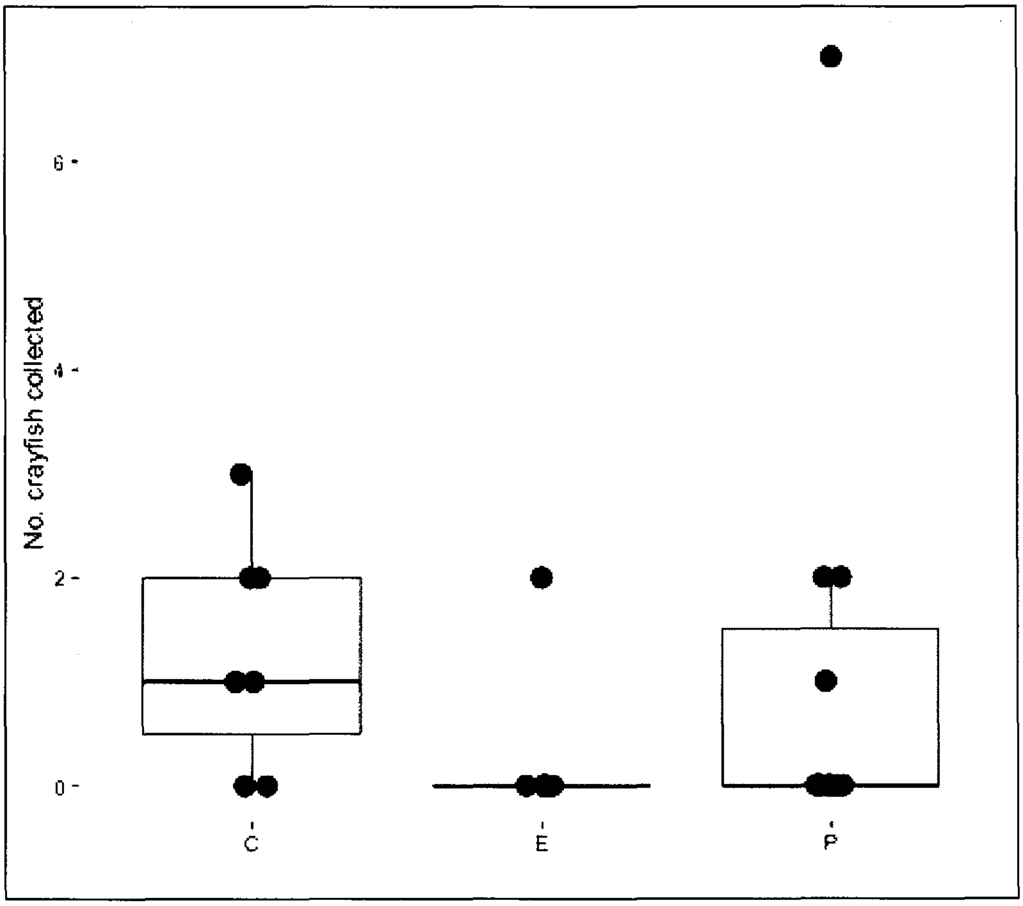

Figure 5. *Number of crayfish detection per site with its catchment type. Plantation labels on x axis: C = Control, E = Eucalypt, P = Pine. Points have been jittered for optical purposes.*

4.3.2 -Power analysis

The ratio of the largest (control) to smallest (eucalypt) mean number of crayfish observed was 4.05, and at least 23 independent streams would have to sampled in each catchment type to achieve an 80% power of detecting this difference with a significance level of 0.05.

4.3.3 - Classification tree

This analysis shows which of the collected variables might be associated with the presence of crayfish. The first node splits the sample into two batches: 14 sites with less than 60% undercut bank to the left (Figure 6), 10 sites with 60% or more to the right. For these latter sites, if the depth was less than 6.65 cm, then no sites had crayfish present; deeper sites were all devoid of crayfish. For the sites with less undercut bank (i.e. the branches on the left of Figure 6), crayfish were present at all sites with conductivities > 111.5 µS/cm, while crayfish presence in more dilute sites was determined by subsequent splits based on the number of submerged logs, log jams and the proportion of the substrate covered by gravels and finer particles as shown below.

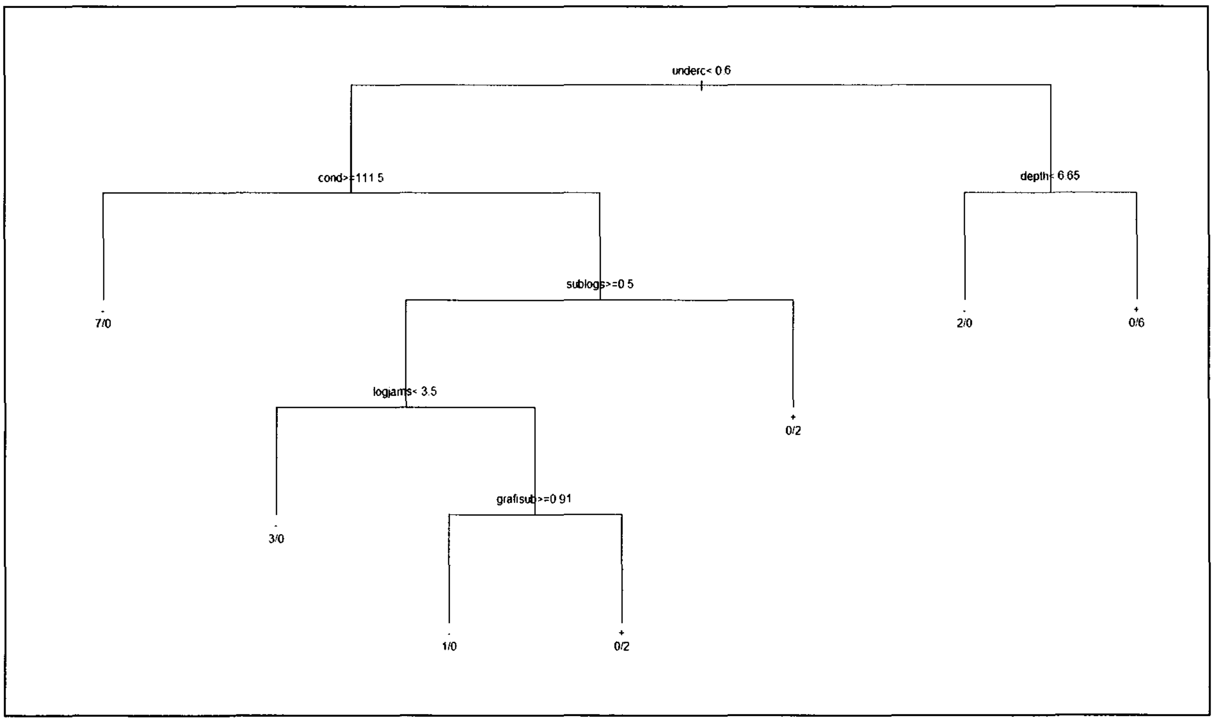

Figure 6. *Classification tree in which each split is called a node and the ends are called leaves. Numbers on the left hand side of each leaf represent absences, while those to the right represent presences. The best variable is listed at each node with a value suggesting a threshold, where values falling above or below this value lead to either a new node or terminate in a presence or absence.*

When conductivity is lower than 111.5 and there are more submerged logs, two sites had crayfish, while if there are no submerged logs, and the number of log jams is < 3.5 and cover by gravels and fine substrates is higher than 91%, then two sites had crayfish present; all other combinations of these variables resulted in sites with no crayfish. The purpose of this classification tree was to explore the data to identify potentially useful variables to retain in future surveys. Inspection of more detailed output from 'rpart' shows variables which are nearly as good as those in Figure 6 in predicting the presence of crayfish for each node, and these are listed in Table 6.

	Node 1	Node 2	Node 3	Node 4	Node 5	Node 6
Top 3 variables at each node	underc	cond	depth	sublogs	logjams	grafisub
	depth	logjams	bslope	logjams	siltsc	siltsc
	siltsc	orgli	logjams	grafisub	canshade	orgli

Table 6. Summary of competing variables for best place at each node in the classification tree. It is noteworthy to mention logjams having 4 appearances as a top 3 variable and is also a habitat creating feature for crayfish; similar to undercut. Refer to appendix for names of abbreviations.

4.4 - Discussion

Based on the outcomes of this study, two possible interpretations can be proposed. Either there really is no plantation effect and the small sample size constitutes a small part of the larger picture. Bailie & Neary (2015) state that water quality in mature *Pinus radiata* plantations is of high quality. If this were also the case in Tasmania, it would suggest there is indeed little effect. Or plantation type affects crayfish abundance and either the sample size used was too small and/or the methods used failed, to appropriately address issues such as sedimentation. Harvesting may alter sediment and woody debris input Bailie & Neary (2015). Whether a plantation is mature or harvested frequently differentially can alter habitat quality and crayfish abundance. This remains to be investigated, preferably with before/after studies.

The power analysis revealed that, if there is a plantation effect as large as the largest difference observed, then about 23 sites would be required to achieve a robust sample size. There lies inherent difficulty in constructing a new survey with this many sites, especially considering that an overall 69 sites would be required for two comparative treatment types plus a control group. A further difficulty stems from the geographical challenge of pairing control sites with close by plantations to have better comparison between treatment and control streams. In addition, MaxEnt predicted a low suitability for the vast majority of available stream sections which lowers the amount of suitable samples.

In light of these seemingly overwhelming difficulties, it is necessary to initiate steps to improve the success of future studies which address the same research question.

Firstly, predictions of low-suitability streams must be re-assessed. Personal communication with Dr. Laurence Cook (*personal communcation*), a crayfish expert, indicated that *A. gouldi* can tolerate stream intermittency. Similar observations were described by A/Prof. Leon Barmuta in the related taxon *Euastacus* in Victoria, Australia (*personal communication*). Therefore, surveying such low-suitability sites could help to improve the model and potentially provide a few more samples to work with. However, as long as *A. gouldi* abundance in intermittent streams remains uncovered in published scientific literature, it is recommended to focus surveys of permanent streams.

Secondly, if considering pooling north-west and north-east Tasmanian streams, it must first be assessed whether it is safe to compare streams between these two, contrasting regions. The current MaxEnt prediction estimates that streams in the North-east are less suitable than the west. This might be as a result of different dominating geology types, lower rainfall and accumulated mean annual run-off (Chapter 3) and a greater influence of historic tin mining. These factors could confound the effect of plantation types and hence direct comparison between the regions.

Thus, when continuing to research crayfish abundance in relation to forestry type, the recommended option, to maximize available samples in light of geographical constraints, is to aim for a sample size of 15-18 per tested group in one region (north-west Tasmania or north-east).

Sample size is not the only factor which requires rethinking: because catchments differed markedly in terms of vegetation composition and sedimentation, better methods of quantifying these variables are needed. For example, the plantation streams surveyed had streamside reserves in place that could reduce the impacts associated with logging on streams. However, instead of being characterized by a mixture of native mosses, ferns, bushes and trees, these buffer zones were often overrun with blackberry. Pines were also observed to have spread into streamside reserves; even in eucalypt plantations. It is unclear whether these originated from previously existing pine plantations or whether they invaded via down-stream dispersal. Regardless, it is unknown whether their invasion could correlate with an absence of crayfish. Therefore, a more in-depth analysis of the effect of riparian vegetation on stream characteristics should be considered in the future.

Another area which should receive more attention is sedimentation. The literature frequently cites it to have the capability to affect crayfish abundance negatively (e.g. Davies et al 2005a, Horwitz 1994). Juvenile crayfish might be more vulnerable to sedimentation because they inhabit smaller streams where fast flowing water can remove sediment and make interstices around rocks and logs available for shelter. If sediment impedes stream flow and the in-stream habitat fills with sediment, then these shelters are lost. Research has shown that when space is limiting, inter-specific competition and aggression increases (Baird *et al.* 2006) which may result in a decreased abundance.

Contrasting this notion, observational evidence from this survey, suggests that older crayfish may become more tolerant towards sedimentation; given that other forms of shelter are present. Sub-adult and adult *A. gouldi* were observed crawling over an extensive fine sediment blanket at 'Site K'. The presence of submerged logs and a high proportion of undercut banks may have represented compensatory refuges for these animals. Research suggests that when placed in sub-optimal conditions, crayfish resort to occupying low quality shelters including trash (Adams 2013); therefore, sedimentation may play a lesser role if other refugia are present. Another site, similar to 'Site K' in the West Gawler region, showed a similar response with two larger crayfish observed traversing fine sediment (7 and 7.7 cm respectively).

It is thus advisable to separate juveniles' and adults' use of habitat and perform separate analyses. However, this discrimination might be influenced strongly by detectability. For instance, juvenile crayfish might be much harder to detect in deeper streams, while it might be easier to detect dark crayfish on a lighter underground where fine sediment predominates. This could introduce bias towards certain sites in the data. Thus occupancy modelling would be a valuable tool to assist this kind of research.

Further adjustments variable collection need to be made. In this study, for instance, quantifying sedimentation occurred through measuring the proportion of embedded rocks, silt cover estimate and quadrat sampling. However, none of these measures seemed to correlate with crayfish abundance in plantations. The inability to effectively measure sedimentation across a stream reach with the employed methods probably helped to blur the effect of sedimentation on crayfish abundance. For example, the proportion of embedded rocks was thought to be a better measure of sedimentation than estimation or using the quadrat. However, when rocks are completely buried by sediment, which was likely the case at 'Site K', the embeddedness percentage would be 0%, which is indicative of no sedimentation and is thus incorrect. Estimating sedimentation by using fewer levels (here low, medium and high were used with 33.3% intervals) across the reach does not account well for patchy sedimentation, where gaps in sediment cover could provide space for juvenile crayfish. Such measures need to be adjusted to be more precise. Without improving sediment measurements to be representative of what is observed in the field, it would be inherently difficult to model the effect of sedimentation on crayfish abundance in plantations.

Lastly, the case is made to assess other possible variables that might not only be associated with crayfish presence, but with other factors that have the potential to enhance sedimentation and other potentially important environmental variables. This would require detailed GIS and field-collected knowledge of soil erodibility, soil composition, but also plantation history. The importance of the latter factor became revealed at one of the Gog Range sites, where sedimentation intensity differed markedly between the upstream re-planted area and the down-stream, recent clear-felled coup. In addition, upstream land-use and conversion history from agricultural or other silvicultural landscapes to the existing state should be incorporated into the study design if this information were to become available.

In summary, if the sample size requirements are met and research methods can more accurately discriminate sites in terms of sedimentation intensity, any new hypothesis developed on this topic will have more explanatory power. Both short and long-term field studies which relate to *A. gouldi* occupancy (because of imperfect detection), thermal tolerance, species movement and habitat use will ultimately help to answer how contrasting plantations types correlate with *A. gouldi* abundance.

Chapter 5 – Closing statement

This study showed little evidence for a statistically significant effect of plantation type on the abundance of the giant Tasmanian freshwater crayfish. On the other hand, this study effectively demonstrated that combining species distribution modelling and then abundance assessments in a forestry setting is a useful alternative to improve site selection. Although no obvious distributional patterns were observed, some evidence suggests that this species is not as sensitive towards environmental stressors as currently presumed. For instance, Chapter 1 showed that loose sand sediment was still a moderate predictor of crayfish presence; hence this might occur in quite different streams well provided that other meso-habitat variables are present. Most crucially, however, presence seems to be linked to the amount of water in a river. Although intermittency may be tolerated to some extent, a greater abundance should be observed in permanent streams.

It was also recommended to build on the findings by this study and roughly double the sample size. Doing so might reveal a pattern that went undetected with the used sample size. In addition, the classification tree might have singled out some important meso-habitat variables which would be worth exploring in the future.

However, without the addition of supplementary information about this species, it will be difficult to interpret the outcomes of any similar survey. Long-term studies are currently unavailable, therefore, occupancy modelling seems to be a valuable tool for consideration in the design of subsequent studies. This can assist in quantifying seasonal abundance or different habitat use at various life-stages. Such knowledge would benefit the outcomes of any survey trying to assess the influence of forestry practices on streams.

Lastly, better ways of quantifying sediment and riparian buffer zones are required. These two factors seem to most immediately affect meso-habitat and might pose a problem for juveniles. Contributors to the NVA or similar databases should also provide information on size, sex, but also a rough estimate of some meso-habitat variables, such as rock cover and sedimentation. There are numerous ways in which such research can be improved. Therefore, to guide future decision, this study has shed light on some of the factors which will require revision in the upcoming years.

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Appendix - Instructions

GIS environmental variables used are found on page 67.

Definitions of field collected variables on page 68-70.

Variable	Shortened name & type (Categorical or continuous)	Units	Agency	Description
Stream order	stream_order (cat)	class	CFEV	displays stream order, increasing with down stream branching
Elevation	rs_elevave (cont)	meters	CFEV	mean height for river section above sea level
Accumulated catchment area	Acccatchment area (cont)	km^2	CFEV	cumulative catchment sizes for downstream river sections. Correlated with mean annual run-off, therefore this is omitted from analysis. The two could be used interchangeably
Accumulated normal mean annual run-off	Accnmmar (Cont)	ML km^2 y^{-1}	CFEV	cumulative run-off estimate for river section (increases downstream) Correlated with accarea.
Slope	rs_slope (cont)		CFEV	river slope at river section
Tasmanian Vegetation 3.0	tasveg_gfc (cat)	class	DPIPWE	categories are: 1 = Agricultural, urban, exotic vegetation 2 = Dry eucalypt and woodland 3 = Highland treeless vegetation 4 = Native grassland 5 = Non-eucalypt forest and woodland 6 = Other natural environments (= 7 = Rainforest and related scrub 8 = Saltmarsh and wetlands 9 = Scrub heathland and coastal complexes 10 = Wet eucalypt forest and woodland 11 = Moorland, sedgeland and peatland 12 = Verified and unverified plantations for silviculture
Geology (1:250k resolution)	geology_merged_geocodes (cat)	class	FPA	1 = Schist and siliceous rocks 2 = Basalt and dolerite 3 = Other 4 = Quartzose sediments / mud-/siltstones 5 = loose sand and fine sediments
Mean annual rainfall	rain_annual (cont)	mm per year	BoM	Mean annual rainfall in Tasmania, 2014
Mean annual temperature	mean_temp (cont)	degrees Celsius	BoM	Mean annual temperature in Tasmania
solar radiation	solar_radiation (cont)	hours	BoM	Mean hours of sunlight per day
Sediment Input	sed_input (cont)	rating	CFEV	based on a continuous score betwee 0 and 1, where 0 is high sedimentation and 1 is low sedimentation
Catchment disturbance	Catchdist (cont)	rating	CFEV	based on a continuous score betwee 0 and 1, where 0 is high disturbance and 1 is undisturbed

Name	* = plant ation only	Unit	Method	Definition
Env. variables				
algal		%	Quadrat	Averaged algal cover for 2 separate quadrat measurements
all rocks		count	Counting	Rocks turned over + embedded rocks
b_slope		category	Optical classification	1 = flat, 2= medium slopes on both sides or one side flat and other site steep, 3 = steep
Bedr		%	Quadrat	Averaged bedrock cover for 2 separate quadrats
bld_cbbl		%	Quadrat	Averaged boulder and cobble cover for 2 separate quadrats; most prefarable type for juveniles to hide under
con_shade		%	Cover of ring	In-field developed way of measuring shading. This involved taking a picture through a ring (formed by thumb and index finger and held about 10cm away and vertical; and estimate what proportion of ring is covered by leaves.
cond		µS/cm	Temperature/Conductivity probe	Estimation of water conductivity
depth		cm	Measuring tape	Stream water depth
dist		m	Measuring tape	Distance sampled in chunks of 50m
duration		hrs	Timer	Aimed for 2 hrs per site
embed		%	Counting	Measurement of sedimentation and embedded (unsuitable) rocks for juvenile crayfish

gra_fi_sub		%	Quadrat	Averaged gravel and fine substrate (sand, but not silt) cover for 2 separate quadrat measurements
logjams		count	Counting	Counting number of logjams
long/lat		Eastings/Northings, GDA94 Z55G	GPS Etrex 20	Record of coordinates
moss		%	Quadrat	Averaged moss cover for 2 separate quadrats
orgli		%	Quadrat	Averaged organic litter cover for 2 separate quadrat measurements
pH		pH	pH paper, probe unavailable	Measurement of water pH
plant	*	category	Classification	1=Control, 2=eucalypt,3=pine
pool		%	Based on distance measurement	Proportion stream = pool
riffle		%	Based on distance measurement	Proportion of stream reach = riffle
rip_zone	*	m	Measuring tape	Measurement of width of riparian buffer zone near plantations
rock_num		count	Counting	Number of turned over rocks with interstices
run		%	Based on distance measurement	Proportion of stream reach = run
silt_sc		%	Estimate	Silt score, where: low sedimentation = 0-33%, mid=33-66%, high = > 66%
sub_logs		count	Counting	Submerged logs in stream reach
time		hrs	Watch	Record of start/end of survey
trap_num		count	Placing baited double ring-net traps	Number of traps deployed at site
underc		%	Estimate	Proportion of undercut banks at site

veg_ht		category	Estimate	Vegetation height, 1 = low (ferns, grasses and small bushes), 2 = small trees and large bushes (up to 5m, including blackberry thickets), 3=tall (trees larger than 5m)
width		cm	Measuring tape	Wetted width of stream
Comment	*			includes information to better describe observed differences in riparian vegetation composition
Crayfish Variables				
Cray_num		count	Detection	Number of crayfish seen at a site
Cray_pres		category	Detection	1=presence,0=absence
cpl		mm but converted to cm	Vernier caliper	Carapace length of crayfish
sex		category	Optical differentiation	juv = indistinguishable sex, linked to size/age, male = sex structures on 2nd last pair of walking legs, female = sex structures on last pair of walking legs